(FILE 'HOME' ENTERED AT 11:41:46 ON 08 APR 2002)

FILE 'BIOSIS, CABA, CAPLUS, EMBASE, LIFESCI, MEDLINE, SCISEARCH, USPATFULL, JAPIO' ENTERED AT 11:42:13 ON 08 APR 2002

L1	1	S CHOLERA B PEPTIDE FRAGMENTS
L2	28	S CHOLERA B AND FRAGMENTS
L3	5	S GM-1 BINDING ACTIVITY
L4	2	S L3 AND MUTANTS
L5	48	S WILLIAMS, NEIL A/AU
L6	34	DUP REM L5 (14 DUPLICATES REMOVED)
L7	104	S HIRST, TIMOTHY R/AU
L8	67	DUP REM L7 (37 DUPLICATES REMOVED)

=>

L4 ANSWER 1 OF 2 USPATFULL

ACCESSION NUMBER:	2001:194402 USPATFULL		
TITLE:	Therapeutic agents		
INVENTOR(S):	Williams, Neil Andrew, Axbridge, Great Britain Hirst, Timothy Raymond, Clevedon, Great Britain Nashar, Toufic Osman, Bristol, Great Britain		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001036917	A1	20011101
APPLICATION INFO.:	US 2001-867914	A1	20010530 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1997-999458, filed on 29 Dec 1997, PENDING		
	NUMBER		DATE
PRIORITY INFORMATION:	GB 1995-13733		19950705
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	MARY M. KRINSKY, Ph. D., J.D., PATENT ATTORNEY, 79 TRUMBULL STREET, NEW HAVEN, CT, 06511		
NUMBER OF CLAIMS:	46		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	28 Drawing Page(s)		
LINE COUNT:	2507		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 2 OF 2 USPATFULL

ACCESSION NUMBER:	2001:152481 USPATFULL		
TITLE:	Therapeutic agents and autoimmune diseases		
INVENTOR(S):	Williams, Neil Andrew, 16 Old Coach Road, Cross, Axbridge, Somerset, United Kingdom BS26 2EF Hirst, Timothy Raymond, 30 Albert Road, Clevedon, North Somerset, United Kingdom BS21 7RR Nashar, Toufic Osman, c/o University of Bristol, School of Medical Services University Walk, Bristol, United Kingdom BS8 1TD		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6287563	B1	20010911
APPLICATION INFO.:	US 1997-999458		19971229 (8)
	NUMBER		DATE
PRIORITY INFORMATION:	GB 1995-13733		19950705
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Borin, Michael		
LEGAL REPRESENTATIVE:	Krinsky, Mary M.		
NUMBER OF CLAIMS:	7		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	14 Drawing Figure(s); 11 Drawing Page(s)		
LINE COUNT:	1328		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=>

8 ANSWER 1 OF 67 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
AN 2002:224794 CAPLUS
TI Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells
AU Soriani, Marco; Bailey, Lorna; **Hirst, Timothy R.**
CS Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, UK
SO Microbiology (Reading, United Kingdom) (2002), 148(3), 667-676
CODEN: MROBEO; ISSN: 1350-0872
PB Society for General Microbiology
DT Journal
LA English
AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a no. of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homolog of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1.alpha. and IL-1.beta. and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor EtxB was able to induce cytokine secretion. The behavior of Ctx and CtxB was very similar to that of Etx and EtxB, resp. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.
RE.CNT 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 67 MEDLINE
AN 2002158646 IN-PROCESS
DN 21887411 PubMed ID: 11890554
TI New insights into the structure-function relationships and therapeutic applications of cholera-like enterotoxins.
AU **Hirst Timothy R**; Fraser Sylvia; Soriani Marco; Aman A Tholib; de Haan Lolke; Hearn Arron; Merritt Ethan
CS Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, UK.. t.r.hirst@bristol.ac.uk
SO INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, (2002 Feb) 291 (6-7) 531-5.
Journal code: 100898849. ISSN: 1438-4221.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020314
Last Updated on STN: 20020314
AB Cholera toxin and E. coli heat-labile enterotoxin are structurally homologous proteins comprised of an enzymatically active A-subunit and five B-subunits that bind with high affinity to GM1-ganglioside receptors found on the surface of mammalian cells. The B-subunits have long been thought of simply as trafficking vehicles that trigger entry and subsequent delivery of the 'toxic' A-subunit into cells. Indeed, such is the capacity of the B-subunits to enter cells, that they have been developed as generic carriers for attachment and delivery of a variety of peptides into mammalian cells. However, the B-subunits also appear to possess discrete 'signalling functions', that induce both transcription factor and cell activation. These are thought to be directly responsible for the potent immunomodulatory properties of the B-subunits, and have resulted in their use as adjuvants and as agents to suppress inflammatory immune disorders. The relationship between the signalling properties of

the B-subunits and their capacity to act as trafficking vehicles has remained unclear. In an effort to understand the structural requirements for these two functions, a set of mutant B-subunits, with amino acid substitutions at position His-57, have been generated and studied. Importantly, such mutant B-subunits retain an ability to bind with high affinity to GM1 and to traffic into cells, but have entirely lost their capacity to activate immune cell populations. Thus, while binding via GM1 appears to be sufficient to trigger cellular uptake it is not sufficient to activate signal transduction. The His-57 region is therefore speculated to be actively engaged in triggering signalling events, possibly via cognate interaction with other cell surface molecules.

L8 ANSWER 3 OF 67 MEDLINE
AN 2002193656 IN-PROCESS
DN 21924257 PubMed ID: 11926133
TI Bacterial toxins as versatile delivery vehicles.
AU de Haan Lolke; **Hirst Timothy R**
CS University of Bristol, Department of Pathology & Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK.
SO Curr Opin Drug Discov Devel, (2002 Mar) 5 (2) 269-78.
Journal code: 100887519. ISSN: 1367-6733.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020404
Last Updated on STN: 20020404
AB An ability to deliver macromolecules into the intracellular compartments of mammalian cells offers enormous potential for development of new therapeutics directed against intracellular targets. Unfortunately, most peptides or proteins are too large to enter the cell cytosol unaided, and any uptake that does occur primarily results in their entry into lysosomes for degradation. However, one group of proteins that possesses an inherent capacity to interact with and enter mammalian cells are bacterial toxins. These are being developed as efficient vehicles for the attachment and intracellular delivery of other macromolecules, including peptides, proteins and DNA. To date, most studies have concentrated on the delivery of immunological epitopes into the endogenous major histocompatibility class I (MHC-I) pathway for development of antiviral or anticancer vaccines. However, opportunities to use toxins to modulate inflammatory autoimmune disorders and cell-specific targeting of DNA for gene therapy illustrates the versatility of toxin molecules as delivery vehicles.

L8 ANSWER 4 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:538538 BIOSIS
DN PREV200100538538
TI A cholera toxin B-subunit variant that binds ganglioside GM1 but fails to induce toxicity.
AU Rodighiero, Chiara; Fujinaga, Yukako; **Hirst, Timothy R.**; Lencer, Wayne I. (1)
CS (1) GI Cell Biology, Children's Hospital, 300 Longwood Ave., Boston, MA, 02115: lencer@tch.harvard.edu USA
SO Journal of Biological Chemistry, (October 5, 2001) Vol. 276, No. 40, pp. 36939-36945. print.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB Entry of cholera toxin (CT) into target epithelial cells and the induction of toxicity depend on CT binding to the lipid-based receptor ganglioside GM1 and association with detergent-insoluble membrane microdomains, a function of the toxin's B-subunit. The B-subunits of CT and related Escherichia coli toxins exhibit a highly conserved exposed peptide loop

(Glu51-Ile58) that faces the cell membrane upon B-subunit binding to GM1. Mutation of His57 to Ala in this loop resulted in a toxin (CT-H57A) that bound GM1 with high apparent affinity, but failed to induce toxicity. CT-H57A bound to only a fraction of the cell-surface receptors available to wild-type CT. The bulk of cell-surface receptors inaccessible to CT-H57A localized to detergent-insoluble apical membrane microdomains (lipid rafts). Compared with wild-type toxin, CT-H57A exhibited slightly lower apparent binding affinity for and less stable binding to GM1 in vitro. Rather than being transported into the Golgi apparatus, a process required for toxicity, most of CT-H57A was rapidly released from intact cells at physiologic temperatures or degraded following its internalization. These data indicate that CT action depends on the stable formation of the CT B-subunitcntdotGM1 complex and provide evidence that GM1 functions as a necessary sorting motif for the retrograde trafficking of toxin into the secretory pathway of target epithelial cells.

L8 ANSWER 5 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2001:397621 BIOSIS
DN PREV200100397621
TI Escherichia coli enterotoxin B subunit triggers apoptosis of CD8+ T cells by activating transcription factor c-Myc.
AU Soriani, Marco; Williams, Neil A.; **Hirst, Timothy R. (1)**
CS (1) Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD: t.r.hirst@bristol.ac.uk UK
SO Infection and Immunity, (August, 2001) Vol. 69, No. 8, pp. 4923-4930. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB Heat-labile enterotoxin from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile enterotoxin (EtxB) was found to induce the selective death of CD8+ T cells, suggesting that EtxB may trigger activation of proapoptotic signaling pathways. Here we show that EtxB treatment of CD8+ T cells but not of CD4+ T cells triggers the specific up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for EtxB-mediated apoptosis. Myc activation was also associated with an increase of IkappaBalpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8+ T cells were pretreated with inhibitors of IkappaBalpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both EtxB-induced apoptosis and Myc expression. Further, a non-receptor-binding mutant of EtxB, EtxB(G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that EtxB is a signaling molecule that triggers activation of transcription factors involved in cell survival.

L8 ANSWER 6 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:303036 BIOSIS
DN PREV200100303036
TI Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its closely related homologue, the B subunit of cholera toxin.
AU Millar, Douglas G. (1); **Hirst, Timothy R.**; Snider, Denis P.
CS (1) Department of Medical Biophysics, Ontario Cancer Institute, 610 University Ave., Room 8-318, Toronto, ON, M5G 2M9: dmillar@uhnres.utoronto.ca Canada

SO Infection and Immunity, (May, 2001) Vol. 69, No. 5, pp. 3476-3482. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB Although cholera toxin (Ctx) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and CtxB. EtxB was found to be a more potent adjuvant than CtxB, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and CtxB have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L8 ANSWER 7 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 2001:317777 CAPLUS
DN 134:362569
TI Cholera toxin and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments
AU Millar, Douglas G.; **Hirst, Timothy R.**
CS Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK
SO Cellular Microbiology (2001), 3(5), 311-329
CODEN: CEMIF5; ISSN: 1462-5814
PB Blackwell Science Ltd.
DT Journal
LA English
AB Cholera toxin (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrheal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrheagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. Treatment of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-d. compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-pos. compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such "antigen depots" could contribute to the immunomodulatory properties of these bacterial virulence determinants.
RE.CNT 99 THERE ARE 99 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 8 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2001:326837 BIOSIS
DN PREV200100326837
TI Is immune cell activation the missing link in the pathogenesis of post-diarrhoeal HUS.
AU Heyderman, Robert S. (1); Soriani, Marco (1); **Hirst, Timothy R. (1)**
CS (1) Dept of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD: r.heyderman@bristol.ac.uk UK
SO Trends in Microbiology, (June, 2001) Vol. 9, No. 6, pp. 262-266. print. ISSN: 0966-842X.
DT Article
LA English
SL English

L8 ANSWER 9 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 2000:431790 BIOSIS
DN PREV200000431790
TI Cholera toxin and related enterotoxins: A cell biological and immunological perspective.
AU de Haan, Lolke; **Hirst, Timothy R. (1)**
CS (1) Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD UK
SO Journal of Natural Toxins, (August, 2000) Vol. 9, No. 3, pp. 281-297. print.
ISSN: 1058-8108.
DT General Review
LA English
SL English
AB Cholera toxin (Ctx) from Vibrio cholerae and the closely related Escherichia coli heat-labile enterotoxin (Etx) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these toxins on gut epithelial cells have revealed important insights into the mechanisms of toxin uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that Ctx and Etx exhibit remarkable immunological properties. When either of these toxins is administered via mucosal routes, it triggers a potent mucosal and systemic anti-toxin immune response. By contrast, local or systemic immunization with other soluble protein antigens usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when Ctx or Etx are mixed with heterologous antigens, they function as adjuvants, leading to stimulation of mucosal responses to the admixed antigen, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these toxins can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the toxins' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest developments within the Ctx/Etx field, with a special emphasis on the cell entry mechanisms and immunomodulatory action of Ctx/Etx and their component subunits.

L8 ANSWER 10 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1999:270440 BIOSIS
DN PREV199900270440

TI Intranuclear delivery of an antiviral peptide mediated by the B subunit of Escherichia coli heat-labile enterotoxin.
AU Loregian, Arianna; Papini, Emanuele; Satin, Barbara; Marsden, Howard S.; **Hirst, Timothy R.**; Palu, Giorgio (1)
CS (1) Institute of Microbiology, University of Padua, 35121, Padua Italy
SO Proceedings of the National Academy of Sciences of the United States of America, (April 27, 1999) Vol. 96, No. 9, pp. 5221-5226.
ISSN: 0027-8424.
DT Article
LA English
SL English
AB We report an intracellular peptide delivery system capable of targeting specific cellular compartments. In the model system we constructed a chimeric protein consisting of the nontoxic B subunit of Escherichia coli heat-labile enterotoxin (EtxB) fused to a 27-mer peptide derived from the DNA polymerase of herpes simplex virus 1. Viral DNA synthesis takes places in the nucleus and requires the interaction with an accessory factor, UL42, encoded by the virus. The peptide, designated Pol, is able to dissociate this interaction. The chimeric protein, EtxB-Pol, retained the functional properties of both EtxB and peptide components and was shown to inhibit viral DNA polymerase activity in vitro via disruption of the polymerase-UL42 complex. When added to virally infected cells, EtxB-Pol had no effect on adenovirus replication but specifically interfered with herpes simplex virus 1 replication. Further studies showed that the antiviral peptide localized in the nucleus, whereas the EtxB component remained associated with vesicular compartments. The results indicate that the chimeric protein entered through endosomal acidic compartments and that the Pol peptide was cleaved from the chimeric protein before being translocated into the nucleus. The system we describe is suitable for delivery of peptides that specifically disrupt protein-protein interactions and may be developed to target specific cellular compartments.

L8 ANSWER 11 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1999:134298 BIOSIS
DN PREV199900134298
TI Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity.
AU Rodighiero, Chiara; Aman, Abu T.; Kenny, Martin J.; Moss, Joel; Lencer, Wayne I.; **Hirst, Timothy R. (1)**
CS (1) Dep. Pathol. Microbiol., Univ. Bristol, Sch. Med. Sci., University Walk, Bristol BS8 1TD UK
SO Journal of Biological Chemistry, (Feb. 12, 1999) Vol. 274, No. 7, pp. 3962-3969.
ISSN: 0021-9258.
DT Article
LA English
AB Cholera toxin (Ctx) and E. coli heat-labile enterotoxin (Etx) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in Etx), as a Y.DEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in Ctx was substituted for that of Etx (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of Etx caused no alteration in toxicity, thus excluding the possibility that the

broader receptor specificity of EtxB is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from Etx A2. This suggests that the reason for the relative potency of Ctx compared with Etx stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L8 ANSWER 12 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1999:237620 BIOSIS
DN PREV199900237620
TI Construction and murine immunogenicity of recombinant Bacille Calmette Guerin vaccines expressing the B subunit of Escherichia coli heat labile enterotoxin.
AU Hayward, Christopher M. M.; O'Gaora, Peadar; Young, Douglas B.; Griffin, George E.; Thole, Jelle; **Hirst, Timothy R.**; Castello-Branco, Luiz R. R.; Lewis, David J. M. (1)
CS (1) Division of Infectious Diseases, Saint George's Hospital Medical School, London, SW17 0RE UK
SO Vaccine, (March, 1999) Vol. 17, No. 9-10, pp. 1272-1281. ISSN: 0264-410X.
DT Article
LA English
SL English
AB Three recombinant strains of Mycobacterium bovis Bacille Calmette Guerin (rBCG) were prepared in which the immunogenic B subunit of human Escherichia coli heat labile enterotoxin (LT-Bh) was expressed either as a cytoplasm protein, a cell wall associated lipoprotein or a secreted protein. Intraperitoneal immunisation of mice with these rBCG induced IgG and IgA antibodies to LT-Bh and shifted the serum IgG subclass response to subsequent challenge with purified LT-Bh from IgG1 to an IgG2a. Oral administration of recombinant BCG induced mucosal and serum IgA antibodies to LT-Bh which peaked four months after immunisation. Antibody responses were greater when LT-Bh was expressed as a secreted protein or lipoprotein rather than in the cytoplasm. Oral vaccination with recombinant BCG may be an effective approach, particularly to induce mucosal IgA and prime for a serum TH1 recall response.

L8 ANSWER 13 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 2000:883742 CAPLUS
DN 135:44842
TI Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic
AU Pitman, Richard S.; **Hirst, Timothy R.**; Williams, Neil A.
CS Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA
SO Recent Research Developments in Immunology (1999), 1(Pt. 2), 497-511 CODEN: RRDIB8
PB Research Signpost
DT Journal; General Review
LA English
AB A review with 59 refs. Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their

activation, differentiation, and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

RE.CNT 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 14 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 1999:397447 BIOSIS
DN PREV199900397447
TI Membrane traffic and the cellular uptake of cholera toxin.
AU Lencer, Wayne I. (1); **Hirst, Timothy R.**; Holmes, Randall K.
CS (1) GI Cell Biology, Combined Program in Pediatric Gastroenterology and Nutrition, Children's Hospital, 300 Longwood Ave., Enders 1220, Boston, MA, 02115 USA
SO Biochimica et Biophysica Acta, (July 8, 1999) Vol. 1450, No. 3, pp. 177-190.
ISSN: 0006-3002.
DT General Review
LA English
SL English
AB In nature, cholera toxin (CT) and the structurally related E. coli heat labile toxin type I (LTI) must breech the epithelial barrier of the intestine to cause the massive diarrhea seen in cholera. This requires endocytosis of toxin-receptor complexes into the apical endosome, retrograde transport into Golgi cisternae or endoplasmic reticulum (ER), and finally transport of toxin across the cell to its site of action on the basolateral membrane. Targeting into this pathway depends on toxin binding ganglioside GM1 and association with caveolae-like membrane domains. Thus to cause disease, both CT and LTI co-opt the molecular machinery used by the host cell to sort, move, and organize their cellular membranes and substituent components.

L8 ANSWER 15 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1999:658083 CAPLUS
DN 132:60181
TI Cholera toxin and Escherichia coli heat-labile enterotoxin
AU **Hirst, Timothy R.**
CS Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK
SO Comprehensive Sourcebook of Bacterial Protein Toxins (2nd Edition) (1999), 104-129. Editor(s): Alouf, Joseph E.; Freer, John H. Publisher: Academic, London, UK.
CODEN: 68GNAV
DT Conference; General Review
LA English
AB A review with many refs. of biogenesis and structure of cholera toxin and related enterotoxins.

RE.CNT 199 THERE ARE 199 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 16 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
AN 1999:459318 BIOSIS
DN PREV199900459318
TI Immune modulation by the cholera-like enterotoxins: From adjuvant to therapeutic.
AU Williams, Neil A. (1); **Hirst, Timothy R. (1)**; Nashar, Toufic O. (1)
CS (1) Dept of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD UK
SO Immunology Today, (Feb., 1999) Vol. 20, No. 2, pp. 95-101.

ISSN: 0167-5699.
DT General Review
LA English

L8 ANSWER 17 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1999:144553 BIOSIS
DN PREV199900144553
TI Receptor mediated apoptosis of CD89+T cells by the B subunits of cholera-like enterotoxins.
AU Pitman, Richard S.; **Hirst, Timothy R.**; Nashar, Toufic O.; Williams, Neil A.
CS Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK
SO Biochemical Society Transactions, (Nov., 1998) Vol. 26, No. 4, pp. S338. Meeting Info.: 666th Meeting of the Biochemical Society Sheffield, England, UK July 29-31, 1998
ISSN: 0300-5127.
DT Conference
LA English

L8 ANSWER 18 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13
AN 1997:313988 BIOSIS
DN PREV199799604476
TI Proteolytic activation of cholera toxin and Escherichia coli labile toxin by entry into host epithelial cells: Signal transduction by a protease-resistant toxin variant.
AU Lencer, Wayne I.; Constable, Carita; Moe, Signa; Rufo, Paul A.; Wolf, Anne; Jobling, Michael G.; Ruston, Steve P.; Madara, James L.; Holmes, Randall K.; **Hirst, Timothy R.**
CS Combined Prog. Pediatr. Gastroenterol. Nutr., Child. Hosp., Enders 1220, 300 Longwood Ave., Boston, MA 02115 USA
SO Journal of Biological Chemistry, (1997) Vol. 272, No. 24, pp. 15562-15568.
ISSN: 0021-9258.
DT Article
LA English
AB Cholera and Escherichia coli heat-labile toxins (CT and LT) require proteolysis of a peptide loop connecting two major domains of their enzymatic A subunits for maximal activity (termed "nicking"). To test whether host intestinal epithelial cells may supply the necessary protease, recombinant rCT and rLT and a protease-resistant mutant CTR192H were prepared. Toxin action was assessed as a Cl- secretory response (Isc) elicited from monolayers of polarized human epithelial T84 cells. When applied to apical cell surfaces, wild type toxins elicited a brisk increase in Isc (80 mu-A/cm-2). Isc was reduced 2-fold, however, when toxins were applied to basolateral membranes. Pretreatment of wild type toxins with trypsin in vitro restored the "basolateral" secretory responses to "apical" levels. Toxin entry into T84 cells via apical but not basolateral membranes led to nicking of the A subunit by a serine-type protease. T84 cells, however, did not nick CTR192H, and the secretory response elicited by CTR192H remained attenuated even when applied to apical membranes. Thus, T84 cells express a serine-type protease(s) fully sufficient for activating the A subunits of CT and LT. The protease, however, is only accessible for activation when the toxin enters the cell via the apical membrane.

L8 ANSWER 19 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14
AN 1997:274651 BIOSIS
DN PREV199799566369
TI Prevention of autoimmune disease due to lymphocyte modulation by the B-subunit of Escherichia coli heat-labile enterotoxin.
AU Williams, Neil A.; Stasiuk, Lisa M.; Nashar, Toufic O.; Richards, Claire M.; Lang, Allison K.; Day, Michael J.; **Hirst, Timothy R.**

CS Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK
SO Proceedings of the National Academy of Sciences of the United States of America, (1997) Vol. 94, No. 10, pp. 5290-5295.
ISSN: 0027-8424.
DT Article
LA English
AB We demonstrate that the receptor binding moiety of Escherichia coli heat-labile enterotoxin (EtxB) can completely prevent autoimmune disease in a murine model of arthritis. Injection of male DBA/1 mice at the base of the tail with type II collagen in the presence of complete Freund's adjuvant normally leads to arthritis, as evidenced by inflammatory infiltration and swelling of the joints. A separate injection of EtxB at the same time as collagen challenge prevented leukocyte infiltration, synovial hyperplasia, and degeneration of the articular cartilage and reduced clinical symptoms of disease by 82%. The principle biological property of EtxB is its ability to bind to the ubiquitous cell surface receptor GM1 ganglioside, and to other galactose-containing glycolipids and galactoproteins. The importance of receptor interaction in mediating protection from arthritis was demonstrated by the failure of a non-receptor-binding mutant of EtxB to elicit any protective effect. Analysis of T cell responses to collagen, in cultures of draining lymph node cells, revealed that protection was associated with a marked increase in interleukin 4 production concomitant with a reduction in interferon gamma levels. Furthermore, in protected mice there was a significant reduction in anti-collagen antibody levels as well as an increase in the IgG1/IgG2a ratio. These observations show that protection is associated with a shift in the Th1/Th2 balance as well as a general reduction in the extent of the anti-type II collagen immune response. This suggests that EtxB-receptor-mediated modulation of lymphocyte responses provides a means of preventing autoimmune disease.

L8 ANSWER 20 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 15
AN 1997:391130 BIOSIS
DN PREV199799690333
TI Structural studies of receptor binding by cholera toxin mutants.
AU Merritt, Ethan A.; Sarfaty, Steve; Jobling, Michael G.; Chang, T.; Holmes, Randall K.; **Hirst, Timothy R.**; Hol, Wim G. J. (1)
CS (1) HHMI, Box 357742, Univ. Washington, Seattle, WA 98195 USA
SO Protein Science, (1997) Vol. 6, No. 7, pp. 1516-1528.
ISSN: 0961-8368.
DT Article
LA English
AB The wide range of receptor binding affinities reported to result from mutations at residue Gly 33 of the cholera toxin B-pentamer (CTB) has been most puzzling. For instance, introduction of an aspartate at this position abolishes receptor binding, whereas substitution by arginine retains receptor affinity despite the larger side chain. We now report the structure determination and 2.3- ANG refinement of the CTB mutant Gly 33 fwdarw Arg complexed with the Gm, oligosaccharide, as well as the 2.2- ANG refinement of a Gly 33 fwdarw Asp mutant of the closely related Escherichia coli heat-labile enterotoxin B-pentamer (LTB). Two of the five receptor binding sites in the Gly 33 fwdarw Arg CTB mutant are occupied by bound G-M1 oligosaccharide; two other sites are involved in a reciprocal toxin:toxin interaction; one site is unoccupied. We further report a higher resolution (2.0 ANG) determination and refinement of the wild-type CTB:G-M1 oligosaccharide complex in which all five oligosaccharides are seen to be bound in essentially identical conformations. Saccharide conformation and binding interactions are very similar in both the CTB wild-type and Gly 33 fwdarw Arg mutant complexes. The protein conformation observed for the binding-deficient Gly 33 fwdarw 4 Asp mutant of LTB does not differ substantially from that seen in the toxin:saccharide complexes. The critical nature of the side chain of residue 33 is apparently due to a limited range of subtle rearrangements available to both the toxin and the

saccharide to accommodate receptor binding. The intermolecular interactions seen in the CTB (Gly 33 fwdarw Arg) complex with oligosaccharide suggest that the affinity of this mutant for the receptor is close to the self-affinity corresponding to the toxin:toxin binding interaction that has now been observed in crystal structures of three CTB mutants.

L8 ANSWER 21 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16
AN 1996:430523 BIOSIS
DN PREV199699161579
TI Assembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin: Kinetics and molecular basis of rate-limiting steps in vitro.
AU Ruddock, Lloyd W.; Coen, Jeremy J. F.; Cheesman, Caroline; Freedman, Robert B.; **Hirst, Timothy R. (1)**
CS (1) Res. Sch. Biosci., Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 32, pp. 19118-19123. ISSN: 0021-9258.
DT Article
LA English
AB The B subunits of Escherichia coli heat-labile enterotoxin (EtxB) and cholera toxin (CtxB) assemble in vivo into exceptionally stable homopentameric complexes, which maintain their quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. Recently, we showed that the simultaneous protonation of two of the COOH-terminal carboxylates in pentameric EtxB was required to cause its disassembly at pH values below 2.0 (Ruddock, L., Ruston, S. P., Kelly, S. M., Price, N. C., Freedman, R. B., and Hirst, T. R. (1995) J. Biol. Chem. 270, 29953-29958). Here, we investigate the influence of environmental parameters on the kinetics of reassembly of acid-generated EtxB monomers in vitro. Such monomers were found to undergo a further acid-mediated conformational change, with an activation energy of 76 +- 2 J cntdot mol-1 cntdot K-1, consistent with isomerization of the cisproline residue at position 93, and which prevented subsequent EtxB reassembly. By using rapid neutralization of acid-generated monomers, a high proportion of the B-subunits adopted an assembly-competent conformation, which resulted in up to 75% of the protein reassembling into a stable pentameric complex, indistinguishable from native EtxB pentamers. The rate-limiting step in reassembly, over a concentration range of 50-200 mu-g/ml, was shown to be due to an intramolecular event, which exhibited a pH dependence with a pK-a of 7.0. Modification of EtxB with amine-specific probes revealed that the protonation state of the NH-2-terminal alanine residue was responsible for the pH dependence of reassembly. The implications of these findings for the biogenesis of Escherichia coli enterotoxin and related enterotoxins in vivo, are considered.

L8 ANSWER 22 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 17
AN 1997:70912 BIOSIS
DN PREV199799370115
TI A pH-dependent conformational change in the B-subunit pentamer of Escherichia coli heat-labile enterotoxin: Structural basis and possible functional role for a conserved feature of the AB-5 toxin family.
AU Ruddock, Lloyd W. (1); Webb, Helen M.; Ruston, Stephen P.; Cheesman, Caroline; Freedman, Robert B.; **Hirst, Timothy R.**
CS (1) Res. Sch. Biosci., Univ. Kent at Canterbury, Canterbury, Kent CT2 7NJ UK
SO Biochemistry, (1996) Vol. 35, No. 50, pp. 16069-16076. ISSN: 0006-2960.
DT Article
LA English
AB The non-covalently associated B-subunit moieties of AB-5 toxins, such as cholera toxin and related diarrheagenic enterotoxins, exhibit exceptional pH stability and remain pentameric at pH values as low as 2.0. Here, we

investigate the structural basis of a pH-dependent conformational change which occurs within the B-5 structure of Escherichia coli heat-labile enterotoxin (EtxB) at around pH 5.0. The use of far-UV CD and fluorescence spectroscopy showed that EtxB pentamers undergo a fully reversible pH-dependent conformational change with a pK-a of 4.9 +- 0.1 (R-2 = 0.999) or 5.13 +- 0.01 (R-2 = 0.999), respectively. This renders the pentamer susceptible to SDS-mediated disassembly and decreases its thermal stability by 18 degree C. A comparison of the pH-dependence of the structural change in EtxB5, with that of a mutant containing a Ser substitution at His 57, revealed that the pK-a of the conformational change was shifted from ca. 5.1 to 4.4. This finding suggests that protonation of the imidazole side chain of His 57 might facilitate disruption of a spatially adjacent salt bridge, located between Glu 51 and Lys 91 in each B-subunit, thus triggering the conformational change in the pentameric structure. The pH-dependent conformational change was found to be inhibited when B-subunits bound to monosialoganglioside, G-M1; and to have no effect on the stability of interaction between A- and B-subunits within the AB-5 complex. This suggests that the conformational change is unlikely to have a direct involvement in toxicity. Conservation of the pH-dependent conformational change in the AB-5 toxin family, combined with the potential exposure of the hydrophobic core of beta-barrel in the monomeric units, leads to the proposal that the conformational change may be the common feature that ensures the secretion of these proteins from the Vibrionaceae.

L8 ANSWER 23 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 18
AN 1996:282100 BIOSIS
DN PREV199699004456
TI PH-dependence of the dithiol-oxidizing activity of DsbA (a periplasmic protein thiol:disulphide oxidoreductase) and protein disulphide-isomerase: Studies with a novel simple peptide substrate.
AU Ruddock, Lloyd W.; **Hirst, Timothy R.**; Freedman, Robert B. (1)
CS (1) Research School Biosciences, Univ. Kent Canterbury, Canterbury, Kent CT2 7NJ UK
SO Biochemical Journal, (1996) Vol. 315, No. 3, pp. 1001-1005. ISSN: 0264-6021.
DT Article
LA English
AB A decapeptide containing two cysteine residues at positions 3 and 8 has been designed for use in monitoring the disulphide bond-forming activity of thiol:disulphide oxidoreductases. The peptide contains a tryptophan residue adjacent to one of the cysteine residues and an arginine residue adjacent to the other. Oxidation of this dithiol peptide to the disulphide state is accompanied by a significant change in tryptophan fluorescence emission intensity. This fluorescence quenching was used as the basis for monitoring the disulphide bond-forming activity of the enzymes protein disulphide-isomerase (PDI) and DsbA (a periplasmic protein thiol: disulphide oxidoreductase) in the pH range 4.0-7.5, where the rates of spontaneous or chemical oxidation are low. Reaction rates were found to be directly proportional to enzyme concentration, and more detailed analysis indicated that the rate-determining step in the overall process was the reoxidation of the reduced form of the enzyme by GSSG. The pH-dependence of the enzyme-catalysed reaction reflected primarily the pK-a of the reactive cysteine residue at the active site of each enzyme. The data indicate a pK-app of 5.6 for bovine PDI and of 5.1 for Vibrio cholerae DsbA.

L8 ANSWER 24 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 19
AN 1996:317111 BIOSIS
DN PREV199699039467
TI Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes.

AU Nashar, Toufic O. (1); Williams, Neil A.; **Hirst, Timothy R.**
CS (1) Res. Sch. Biosciences, Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO International Immunology, (1996) Vol. 8, No. 5, pp. 731-736.
ISSN: 0953-8178.
DT Article
LA English
AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (EtxB) of Escherichia coli heat-labile enterotoxin (Etx) is a potent immunogen in vivo and has profound modulatory effects on EtxB-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by EtxB causes a differential effect on mature CD4+ and CD8+ T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of EtxB to such cultures led to the complete depletion of CD8+ T cells compared with enhanced activation of CD4+ T cells (as measured by expression of CD25 (IL-2R-alpha)). By contrast, addition of a mutant EtxB, EtxB(G33D), which does not bind to GM1, failed to trigger CD8+ T cell depletion. When EtxB was added to isolated non-immune CD8+ lymphocytes rapid, (12-18 h) alterations in nuclear morphology and the appearance of sub-G-0/G-1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. EtxB(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8+ T lymphocytes.

L8 ANSWER 25 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 20
AN 1997:25812 BIOSIS
DN PREV199799325015
TI Use of Vibrio spp. for expression of Escherichia coli enterotoxin B subunit fusion proteins: Purification and characterization of a chimera containing a C-terminal fragment of DNA polymerase from herpes simplex virus type 1.
AU Loregian, Arianna; **Hirst, Timothy R.**; Marsden, Howard S.; Palu, Giorgio (1)
CS (1) Inst. Microbiol., Univ. Padova, via Gabelli 63, 35121 Padua Italy
SO Protein Expression and Purification, (1996) Vol. 8, No. 3, pp. 381-389.
ISSN: 1046-5928.
DT Article
LA English
AB The nontoxic B subunit of Escherichia coli heat-labile enterotoxin (EtxB) is a convenient carrier molecule for the attachment and delivery of heterologous peptides into eukaryotic cells. To evaluate the properties of such EtxB-based fusion proteins an efficient method for their production and purification is required. High-level production and purification of native EtxB has been achieved using heterologous expression and secretion in a marine Vibrio (Amin, T., and Hirst, T. R., 1994, Protein Expression Purif. 5, 198 204). However, the use of this method to isolate EtxB fusion proteins has been precluded because of their susceptibility to degradation by extracellular proteases secreted by members of the Vibrionaceae. In this paper a method is described for production of EtxBpol, comprising the enterotoxin B subunit linked to a 27-residue C-terminal fragment of Pol, the catalytic subunit of DNA polymerase of herpes simplex virus type 1 (HSV-1). Following assessment of the relative efficacy of different Vibrio strains as hosts for EtxBpol expression, the chimera was produced at the highest level of 3.5 mg/liter by cultures of Vibrio sp.60. Addition of 0.3 mm EDTA to the growth medium blocked proteolysis of the secreted EtxB-pol fusion protein, which was then purified to homogeneity using ammonium sulfate fractionation and hydrophobic interaction chromatography, with a

yield of 57%. Purified EtxB-pol reacted with both anti-EtxB and anti-Pol peptide antibodies, and was able to specifically bind UL42, a processivity factor which normally binds to the C-terminal region of HSV-1 Pol. This modified method for expression and purification of EtxB-pol should be of general utility for the preparation of other EtxB-based fusion proteins.

L8 ANSWER 26 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 21
AN 1996:107787 BIOSIS
DN PREV199698679922
TI Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: Receptor binding is essential and induces differential modulation of lymphocyte subsets.
AU Nashar, Toufic O. (1); Webb, Helen M.; Eaglestone, Simon; Williams, Neil A.; **Hirst, Timothy R.**
CS (1) Research School Biosciences, University Kent, Canterbury, Kent CTG2 7NJ UK
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 1, pp. 226-230.
ISSN: 0027-8424.
DT Article
LA English
AB The importance of receptor binding in the potent immunogenicity of Escherichia coli heat-labile enterotoxin B subunit (EtxB) was tested by comparing its immunogical properties with those of a receptor binding mutant, EtxB(G33D). Subcutaneous immunization of EtxB(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type EtxB, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two proteins induced strikingly different effects on lymphocyte cultures in vitro. EtxB, in comparison with EtxB(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma. These data indicate that EtxB exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

L8 ANSWER 27 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:294095 BIOSIS
DN PREV199699016451
TI Biogenesis of cholera toxin and related oligomeric enterotoxins.
AU **Hirst, Timothy R.**
CS Res. Sch. Biosci., Univ. Kent, Canterbury, Kent UK
SO Moss, J. [Editor]; Iglewski, B. [Editor]; Vaughan, M. [Editor]; Tu, A. T. [Editor]. Handbook of Natural Toxins, (1995) Vol. 8, pp. 123-184. Handbook of Natural Toxins; Bacterial toxins and virulence factors in disease. Publisher: Marcel Dekker, Inc. 270 Madison Avenue, New York, New York 10016, USA.
ISBN: 0-8247-9381-1.
DT Book
LA English

L8 ANSWER 28 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 22
AN 1996:67032 BIOSIS
DN PREV199698639167
TI Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin: Molecular basis of pH stability.
AU Ruddock, Lloyd W.; Ruston, Stephen P.; Kelly, Sharon M.; Price, Nicholas C.; Freedman, Robert B.; **Hirst, Timothy R. (1)**
CS (1) Res. Sch. Biosci., Univ. Kent Canterbury, Canterbury, Kent CT2 7NJ UK
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 50, pp. 29953-29958.

ISSN: 0021-9258.
DT Article
LA English
AB The B-subunit pentamer of Escherichia coli heat-labile enterotoxin (EtxB) is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of EtxB has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques. Disassembly of the cyclic pentameric structure of human EtxB occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H+ ion concentration, and with an activation energy of 155 kJ mol-1. A C-terminal deletion mutant, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human EtxB being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of cholera toxin (CtxB), a protein which shows 80% sequence identity with EtxB, exhibits a much lower stability to acid conditions; with disassembly of CtxB occurring below pH 3.9, with an activation energy of 81 kJ mol-1. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to the development of oral vaccines using EtxB and CtxB are considered.

L8 ANSWER 29 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 23
AN 1995:459495 BIOSIS
DN PREV199598473795
TI Generation of a monoclonal antibody that recognizes the amino-terminal decapeptide of the B-subunit of Escherichia coli heat-labile enterotoxin: A new probe for studying toxin assembly intermediates: A new probe for studying toxin assembly intermediates.
AU Amin, Tehmina; Larkins, Audrey; James, Roger F. L.; **Hirst, Timothy R. (1)**
CS (1) Res. Sch. Biosci., Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 34, pp. 20143-20150. ISSN: 0021-9258.
DT Article
LA English
AB Cholera toxin and the related Escherichia coli heat-labile enterotoxin are hexameric proteins comprising one A-subunit and five B-subunits. In this paper we report the generation and characterization of a monoclonal antibody, designated LDS47, that recognizes and precipitates in vivo assembly intermediates of the B-subunit (EtxB) of E. coli heat-labile enterotoxin. The monoclonal antibody is unable to precipitate native B-subunit pentamers, thus making LDS47 a useful probe for studying the early stages of enterotoxin biogenesis. The use of LDS47 to monitor the in vivo turnover of newly synthesized B-subunits in the periplasm of E. coli demonstrated that (i) the turnover of unassembled B-subunits followed an apparent first order process and (ii) it occurred concomitantly with the assembly of native B-pentamers ($k = 0.317 \pm 0.170$ min-1; $t_{1/2} = 2.2$ min). No other proteins were co-precipitated with the newly synthesized B-subunits; a finding that implies that unassembled B-subunits do not stably associate with other periplasmic proteins prior to their assembly into a macromolecular complex. The use of overlapping synthetic peptides corresponding to the entire EtxB polypeptide demonstrated that the epitope recognized by LDS47 is located within the amino-terminal decapeptide of the B-subunit. From the x-ray structural analysis of the toxin (Sixma, T., Kalk, K., van Zanten, B., Dauter, Z., Kingma, J., Witholt, B., and Hol, W. G. J. (1993) J. Mol. Biol. 230, 890-918), this region appears to resemble a curved finger that clasps the adjacent B-subunit. Thus, this region might be expected to be exposed in the unfolded or unassembled subunit, but to become partially buried upon assembly and thus inaccessible to

recognition by the monoclonal antibody.

L8 ANSWER 30 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 24
AN 1995:438061 BIOSIS
DN PREV199598452361
TI Immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB.
AU Nashar, Toufic O. (1); **Hirst, Timothy R.**
CS (1) Res. Sch. Biosci., Univ Kent Canterbury, Canterbury, Kent CT2 7NJ UK
SO Vaccine, (1995) Vol. 13, No. 9, pp. 803-810.
ISSN: 0264-410X.
DT Article
LA English
AB The immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB) is reported. Oral delivery of rEtxB to congenic mice of several different H-2 haplotypes resulted in H-2 dependent serum IgG responses (H-2-d gt H-2-b =H-2-q gt H-2-a gt H-2-k) and a similar spectrum of intestinal IgA responses in those strains tested. Responses to rEtxB and rCtxB were found to be differentially modulated by the H-2 locus, with significant differential effects in H-2-b and H-2-d congenic strains (H-2-d gt H-2-b for rEtxB; H-2-b gt H-2-d for rCtxB). Additionally, it was found that when rEtxB was fed to mice previously primed (orally) with either rEtxB or rCtxB only those mice primed with rEtxB exhibited a booster response. A second booster immunisation with rEtxB in rCtxB-primed mice produced an H-2 dependent spectrum of responses characteristic of those elicited by rEtxB, with the antibodies predominantly directed against rEtxB and not rCtxB. These results indicate that the differential response to rEtxB and rCtxB is set at the T- and B-cell leveL Also, immunoregulation of antibody responses to rEtxB by intra-H-2 I-E in mice transgenic for the entire IE-a-k gene was investigated. No significant difference between responses in transgene-positive and -negative mice was found, suggesting that antigen presentation does not involve I-E, but occurs in the context of I-A. The implications of these results for the design of vaccines against enterotoxigenic E. coli and cholera diarrhoea are discussed.

L8 ANSWER 31 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1995:690703 CAPLUS
DN 123:104417
TI Biogenesis of cholera toxin and related oligomeric enterotoxins
AU **Hirst, Timothy R.**
CS Research School Biosciences, University Kent, Canterbury/Kent, UK
SO Handb. Nat. Toxins (1995), 8(Bacterial Toxins and Virulence Factors in Disease), 123-84
CODEN: HNTOE5
DT Journal; General Review
LA English
AB A review with many refs. Cholera and related diarrheal diseases, cholera toxin and related enterotoxins, toxin gene organization, and biogenesis of cholera toxin and related enterotoxins are discussed.

L8 ANSWER 32 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:232279 BIOSIS
DN PREV199598246579
TI Cloning and expression of Vibrio cholerae dsbA, a gene encoding a periplasmic protein disulphide isomerase.
AU Lowe, Edward D. (1); Freedman, Robert B. (1); **Hirst, Timothy R. (1)**; Barth, Peter T.
CS (1) Research Sch. Biosci., Univ. Canterbury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1995) Vol. 23, No. 1, pp. 64S.
Meeting Info.: 652nd Meeting of the Biochemical Society Canterbury,

England, UK September 6-9, 1994
ISSN: 0300-5127.
DT Conference
LA English

L8 ANSWER 33 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1995:404768 CAPLUS
DN 122:206892
TI Cloning and expression of Vibrio cholerae dsbA, a gene encoding a periplasmic protein disulfide isomerase
AU Lowe, Edward D.; Freedman, Robert B.; **Hirst, Timothy R.**; Barth, Peter T.
CS Res. Sch. of Biosciences, Univ. Canterbury, Canterbury/Kent, CT2 7NJ, UK
SO Biochem. Soc. Trans. (1995), 23(1), 64S
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English
AB The protein disulfide isomerase gene dsbA was cloned from V. cholerae, amplified by PCR, and expressed in Escherichia coli. The dsbA gene was regulated by the phage T7 gene 10 promoter, and its expression was induced by IPTG. The unusual nture of the encoded enzyme as a folding catalyst contg. no tryptophan residues presents an opportunity to study the in vitro folding of tryptophan-contg. protein substates.

L8 ANSWER 34 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 25
AN 1995:232276 BIOSIS
DN PREV199598246576
TI Construction of a fusion protein between B subunit of E. coli heat-labile enterotoxin and the C-terminus of herpes simplex virus-DNA polymerase.
AU Loregian, Arianna (1); Marcello, Alessandro (1); **Hirst, Timothy R.** ; Marsden, Howard S.; Palu, Giorgio
CS (1) Inst. Microbiol., Univ. Padova Italy
SO Biochemical Society Transactions, (1995) Vol. 23, No. 1, pp. 61S.
Meeting Info.: 652nd Meeting of the Biochemical Society Canterbury, England, UK September 6-9, 1994
ISSN: 0300-5127.
DT Conference
LA English

L8 ANSWER 35 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 26
AN 1995:232249 BIOSIS
DN PREV199598246549
TI A pleiotropic secretion mutant of Aeromonas hydrophila is unable to secrete heterologously expressed E. coli enterotoxin: Implication for common mechanisms of protein secretion.
AU Yu, Jun; **Hirst, Timothy R.**
CS Research Sch. Biosci., Univ. Kent, Cantebury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1995) Vol. 23, No. 1, pp. 34S.
Meeting Info.: 652nd Meeting of the Biochemical Society Canterbury, England, UK September 6-9, 1994
ISSN: 0300-5127.
DT Conference
LA English

L8 ANSWER 36 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 27
AN 1994:479508 BIOSIS
DN PREV199497492508
TI Specific inhibition of herpes virus replication by receptor-mediated entry of an antiviral peptide linked to Escherichia coli enterotoxin B subunit.
AU Marcello, Alessandro; Loregian, Arianna; Cross, Anne; Marsden, Howard; **Hirst, Timothy R.**; Palu, Giorgio (1)

CS (1) Inst. Microbiol., Univ. Padova, 35121 Padova Italy
SO Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 19, pp. 8994-8998.
ISSN: 0027-8424.
DT Article
LA English
AB Mimetic peptides capable of selectively disrupting protein-protein interactions represent potential therapeutic agents for inhibition of viral and cellular enzymes. This approach was first suggested by the observation that the peptide YAGAVVNDL, corresponding to the carboxyl-terminal 9 amino acids of the small subunit of ribonucleotide reductase of herpes simplex virus, specifically inhibited the viral enzyme in vitro. Evaluation and use of this peptide as a potential antiviral agent has, however, been thwarted by its failure to inhibit virus replication in vivo, presumably because the peptide is too large to enter eukaryotic cells unaided. Here, we show that the nontoxic B subunit of Escherichia coli heat-labile enterotoxin can be used as a recombinant carrier for the receptor-mediated delivery of YAGAVVNDL into virally infected cells. The resultant fusion protein specifically inhibited herpes simplex virus type 1 replication and ribonucleotide reductase activity in quiescent Vero cells. Preincubation of the fusion protein with soluble GM1 ganglioside abolished this antiviral effect, indicating that receptor-mediated binding to the target cell is necessary for its activity. This provides direct evidence of the usefulness of carrier-mediated delivery to evaluate the intracellular efficacy of a putative antiviral peptide.

L8 ANSWER 37 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 28
AN 1995:86754 BIOSIS
DN PREV199598101054
TI Comparison of the glycolipid-binding specificities of cholera toxin and porcine Escherichia coli heat-labile enterotoxin: Identification of a receptor-active non-ganglioside glycolipid for the heat-labile toxin in infant rabbit small intestine.
AU Teneberg, Susann (1); **Hirst, Timothy R.**; Angstrom, Jonas; Karlsson, Karl-Anders
CS (1) Dep. Med. Biochem. Microbiol., Goteborg Univ., Medicinaregatan 9, S-413 90 Goteborg Sweden
SO Glycoconjugate Journal, (1994) Vol. 11, No. 6, pp. 533-540.
ISSN: 0282-0080.
DT Article
LA English
AB The binding specificities of cholera toxin and Escherichia coli heat-labile enterotoxin were investigated by binding of 125I-labelled toxins to reference glycosphingolipids separated on thin-layer chromatograms and coated in microtitre wells. The binding of cholera toxin was restricted to the GM1 ganglioside. The heat-labile toxin showed the highest affinity for GM1 but also bound, though less strongly, to the GM2, GD2 and GD1b gangliosides and to the non-acid glycosphingolipids gangliotetraosylceramide and lactoneotetraosylceramide. The infant rabbit small intestine, a model system for diarrhoea induced by the toxins, was shown to contain two receptor-active glycosphingolipids for the heat-labile toxin, GM1 ganglioside and lactoneotetraosylceramide, whereas only the GM1 ganglioside was receptor-active for cholera toxin. Preliminary evidence was obtained, indicating that epithelial cells of human small intestine also contain lactoneotetraosylceramide and similar sequences. By computer-based molecular modelling, lactoneotetraosylceramide was docked into the active site of the heat-labile toxin, using the known crystal structure of the toxin in complex with lactose. Interactions which may explain the relatively high toxin affinity for this receptor were found.

L8 ANSWER 38 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

29
AN 1994:379361 BIOSIS
DN PREV199497392361
TI Bacterial and host interactions during the biogenesis, toxicity and immunogenicity of Escherichia coli heat-labile enterotoxin.
AU **Hirst, Timothy R. (1)**; Nashar, Toufic O.; Eaglestone, Simon; Lencer, Wayne I.; Webb, Helen M.; Yu, Jun
CS (1) Res. Sch. Biosciences, Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1994) Vol. 22, No. 2, pp. 306-309.
Meeting Info.: 649th Meeting of the Biochemical Society London, England, UK December 19-21, 1993
ISSN: 0300-5127.
DT Conference
LA English

L8 ANSWER 39 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 30
AN 1994:256239 BIOSIS
DN PREV199497269239
TI Purification of the B-subunit oligomer of Escherichia coli heat-labile enterotoxin by heterologous expression and secretion in a marine vibrio.
AU Amin, Tehmina; **Hirst, Timothy R.**
CS Biol. Lab., Univ., Canterbury, Kent CT2 7NJ UK
SO Protein Expression and Purification, (1994) Vol. 5, No. 2, pp. 198-204.
ISSN: 1046-5928.
DT Article
LA English
AB Heat-labile enterotoxins (Etx) are plasmid-encoded, multimeric proteins produced by certain diarrheagenic strains of Escherichia coli. The nontoxic, receptor-binding B subunit (EtxB) of such toxins may be useful as a component of vaccines against enterotoxigenic E. coli, or as a carrier for the delivery of heterologous epitopes to the mucosal immune system. Here we describe a simple method for the purification of EtxB from a marine vibrio harboring a broad-host range controlled expression vector containing the etxB gene. Induction of EtxB resulted in its specific secretion to the medium, to a concentration of greater than 25 mg/liter of culture. The techniques of ultrafiltration and hydrophobic interaction chromatography were used to purify EtxB to homogeneity from the medium of this organism (with a yield of 60.7%). EtxB-epitope fusion proteins were also successfully expressed and secreted in this marine vibrio, suggesting that this system may be of general use in the preparation of EtxB-based vaccines.

L8 ANSWER 40 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1994:191630 BIOSIS
DN PREV199497204630
TI A secreted lectin from Aeromonas hydrophila with affinity for collagen.
AU Ascencio, Felipe (1); **Hirst, Timothy R.**; Waldstrom, Torkel
CS (1) Dep. Marine Pathol., Cent. Biol. Res., La Paz, BCS 23000 Mexico
SO Journal of Cellular Biochemistry Supplement, (1994) Vol. 0, No. 18 PART A, pp. 68.
Meeting Info.: Keystone Symposium on Molecular Events in Microbial Pathogenesis Santa Fe, New Mexico, USA January 8-14, 1994
ISSN: 0733-1959.
DT Conference
LA English

L8 ANSWER 41 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 31
AN 1994:216497 BIOSIS
DN PREV199497229497
TI Efficient extracellular production of hybrid E. coli heat-labile enterotoxin B subunits in a marine vibrio.
AU Marcello, Alessandro (1); Loregian, Arianna; Palu, Giorgio; **Hirst,**

Timothy R.
CS (1) Inst. Microbiol., Univ. Padua, via Gabelli 63, 35121 Padua Italy
SO FEMS Microbiology Letters, (1994) Vol. 117, No. 1, pp. 47-51.
ISSN: 0378-1097.
DT Article
LA English
AB Escherchia coli heat-labile enterotoxin B subunit (EtxB) has been proposed as a potential protein carrier for the delivery of heterologous peptides to target cells, particularly for the oral delivery of epitopes to the mucosal immune system. In this study, two extensions to the C-terminus of EtxB were genetically engineered that correspond to a well-characterized neutralising epitope of glycoprotein D from herpes simplex virus (EtxB-gD) and to the C-terminal nine amino acids from the 38 kDa subunit of HSV-encoded ribonucleotide reductase (EtxB-R2). Here we describe the extracellular secretion of the two hybrid EtxBs from a marine Vibrio harbouring a broad-host range inducible expression vector containing the hybrid genes. Large amounts of intact fusion proteins (15-20 mg per liter of culture) were secreted into the medium upon induction. These hybrid proteins maintained the receptor-binding activity of the native toxin as well as being cross-reactive with anti-EtxB and anti-heterologous peptide monoclonal antibodies.

L8 ANSWER 42 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 32
AN 1993:206751 BIOSIS
DN PREV199395107976
TI Cloning and active site mutagenesis of Vibrio cholerae DsbA, a periplasmic enzyme that catalyzes disulfide bond formation.
AU Yu, Jun; McLaughlin, Stephen; Freedman, Robert B.; **Hirst, Timothy R. (1)**
CS (1) Biological Lab., Univ. Kent Canterbury, Canterbury, Kent CT2 7NJ UK
SO Journal of Biological Chemistry, (1993) Vol. 268, No. 6, pp. 4326-4330.
ISSN: 0021-9258.
DT Article
LA English
AB Recently, a gene (dsbA) involved in the biogenesis of secreted oligomeric enterotoxins in Vibrio cholerae was described, which encodes an exported protein possessing a -Cys-Pro-His-Cys- motif similar to that found in the active sites of eukaryotic and prokaryotic thiol-disulfide oxidoreductases (Yu, J., Webb, H., and Hirst, T.R. (1992) Mol. Microbiol. 6, 1949-1958). Here, we report the cloning of the dsbA gene of V. cholerae and the demonstration that the encoded periplasmic enzyme has disulfide isomerase-like activity. Oligonucleotide-directed mutagenesis of either of the 2 Cys residues to Ala in the putative active site of DsbA abolished both its isomerase activity and its capacity to promote enterotoxin biogenesis. We conclude that the Cys residues constitute the active site domain of DsbA and are essential for its activity in vivo and in vitro.

L8 ANSWER 43 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 33
AN 1993:157796 BIOSIS
DN PREV199344076596
TI Current progress in the development of the B subunits of cholera toxin and Escherichia coli heat-labile enterotoxin as carriers fot the oral delivery of heterologous antigens and epitopes.
AU Nashar, Toufic O.; Amin, Tehmina; Marcello, Alessandro; **Hirst, Timothy R. (1)**
CS (1) Biological Lab., Univ. Kent Canterbury, Canterbury, Kent CT2 7NJ UK
SO Vaccine, (1993) Vol. 11, No. 2, pp. 235-240.
Meeting Info.: International Conference on Vaccines for Enteric Diseases Cambridge, England, UK April 13-15, 1992
ISSN: 0264-410X.
DT Article
LA English

L8 ANSWER 44 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 34
AN 1993:423407 BIOSIS
DN PREV199345071032
TI A new method for the purification of the B subunit (EtxB) of Escherichia coli heat-labile enterotoxin.
AU Amin, Tehmina; Marcello, Alessandro; **Hirst, Timothy R.**
CS Biol. Lab., Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1993) Vol. 21, No. 2, pp. 213S.
Meeting Info.: 645th Meeting of the Biochemical Society on Phospholipid Translocation, Asymmetry and Membrane Fusion London, England, UK December 15-18, 1992
ISSN: 0300-5127.
DT Article
LA English

L8 ANSWER 45 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:423406 BIOSIS
DN PREV199345071031
TI Analysis of enterotoxin synthesis in a Vibrio cholerae strain lacking DsbA, a periplasmic enzyme involved in disulphide bond formation.
AU Findlay, Gordon; Yu, Jun; **Hirst, Timothy R.**
CS Biol. Lab., Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1993) Vol. 21, No. 2, pp. 212S.
Meeting Info.: 645th Meeting of the Biochemical Society on Phospholipid Translocation, Asymmetry and Membrane Fusion London, England, UK December 15-18, 1992
ISSN: 0300-5127.
DT Article
LA English

L8 ANSWER 46 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1993:424413 CAPLUS
DN 119:24413
TI Analysis of enterotoxin synthesis in a Vibrio cholerae strain lacking DsbA, a periplasmic enzyme involved in disulfide bond formation
AU Findlay, Gordon; Yu, Jun; **Hirst, Timothy R.**
CS Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK
SO Biochem. Soc. Trans. (1993), 21(2), 212S
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English
AB To investigate the events of enterotoxin biogenesis the authors used a simplified system consisting of a vibrio strain with a chromosomal ctx gene deletion harboring the plasmid pMMB107, which encodes only the B-subunit of cholera-like enterotoxin (EtxB). Transposon (TnphoA) mutagenesis of this strain resulted in the identification of a mutant, UKC13::TnphoA.7A (pMMB107) with a 50-fold redn. in the level of the EtxB secretion. TnphoA insertion was found to be in a gene encoding a periplasmic protein with 40% homol. to the recently identified disulfide bond-forming protein (DsbA) of E. coli. To examine the role of DsbA in ExtB biogenesis, the dsbA::TnphoA mutant strain was cultured in minimal medium, pulse-labeled with 35S-Met and the fate of radiolabeled ExtB in periplasmic and medium fractions analyzed by SDS-PAGE and autoradiog. This demonstrated that EtxB was exported to the periplasm in both the mutant and the wild-type strain, but only secreted to the medium in the wild-type strain. The EtxB in the periplasm of the mutant strain was rapidly lost, probably as a result of proteolytic degrdn. This demonstrates that DsbA is not required for translocation of ExtB to the periplasm, but plays an important role in subsequent steps of toxin formation.

L8 ANSWER 47 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE

35
AN 1993:99640 BIOSIS
DN PREV199395054836
TI Intermolecular interactions between the A and B subunits of heat-labile enterotoxin from Escherichia coli promote holotoxin assembly and stability in vivo.
AU Streatfield, Stephen J.; Sandkvist, Maria; Sixma, Titia K.; Bagdasarian, Michael; Hol, Wim G. J.; **Hirst, Timothy R. (1)**
CS (1) Biological Lab., University Kent, Canterbury, Kent CT2 7NJ, Great Britain
SO Proceedings of the National Academy of Sciences of the United States of America, (1992) Vol. 89, No. 24, pp. 12140-12144.
ISSN: 0027-8424.
DT Article
LA English
AB Cholera toxin and the related heat-labile enterotoxin (LT) produced by Escherichia coli consist of a holotoxin of one A subunit and five B subunits (AB-5). Here we investigate the domains of the A subunit (EtxA) of E. coli LT which influence the events of B-subunit (EtxB) oligomerization and the formation of a stable AB-5 holotoxin complex. We show that the C-terminal 14 amino acids of the A subunit comprise two functional domains that differentially affect oligomerization and holotoxin stability. Deletion of the last 14 amino acids (-14) from the A subunit resulted in a molecule that was significantly impaired in its capacity to promote the assembly of a mutant B subunit, EtxB191.5. In contrast, deletion of the last four amino acids (-4) from the A subunit gave a molecule that retained such a capacity. This suggest that C-terminal residues within the -14 to -4 region of the A subunit are important for promoting the oligomerization of EtxB. In addition, we demonstrate that the truncated A subunit lacking the last 4 amino acids was unable to form a stable AB-5 holotoxin complex even though it promoted B-subunit oligomerization. This suggests that the last 4 residues of the A subunit function as an "anchoring" sequence responsible for maintaining the stability of A/B subunit interaction during holotoxin assembly. These data represent an important example of how intermolecular interactions between polypeptides in vivo can modulate the folding and assembly of a macromolecular complex.

L8 ANSWER 48 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1992:528012 CAPLUS
DN 117:128012
TI A homolog of the Escherichia coli DsbA protein involved in disulfide bond formation is required for enterotoxin biogenesis in Vibrio cholerae
AU Yu, Jun; Webb, Helen; **Hirst, Timothy R.**
CS Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK
SO Mol. Microbiol. (1992), 6(14), 1949-58
CODEN: MOMIEE; ISSN: 0950-382X
DT Journal
LA English
AB A strain of V. cholerae, which had been engineered to express high levels of the non-toxic B subunit (EtxB) of E. coli heat-labile enterotoxin, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amt. of EtxB produced. The loci disrupted by TnphoA in the two mutant derivs. were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulfide isomerase, and DsbA (a periplasmic protein required for disulfide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivs. was found to restore enterotoxin formation, while expression of Etx or EtxB in a dsbA mutant of E. coli

confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each EtxB subunit contains a single intramol. disulfide bond, a transient intermol. interaction with DsbA occurs during toxin subunit folding which catalyzes formation of the disulfide in vivo.

L8 ANSWER 49 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1992:149603 CAPLUS
DN 116:149603
TI Development of an immunoassay using recombinant maltose-binding protein-STa fusions for quantitating antibody responses against STa, the heat-stable enterotoxin of Escherichia coli
AU Aitken, Robert; **Hirst, Timothy R.**
CS Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK
SO J. Clin. Microbiol. (1992), 30(3), 732-4
CODEN: JCMIDW; ISSN: 0095-1137
DT Journal
LA English
AB A set of fusion proteins contg. heat-stable enterotoxin (STa) and maltose-binding protein were engineered. These mols. were readily purified and used as solid-phase antigens in an ELISA to monitor anti-STa responses in mice immunized with a recombinant vaccine composed of STa and the B subunit of heat-labile enterotoxin.

L8 ANSWER 50 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1992:402474 CAPLUS
DN 117:2474
TI Expression of the B subunit of Escherichia coli heat-labile enterotoxin in a marine Vibrio and in a mutant that is pleiotropically defective in the secretion of extracellular proteins
AU Leece, Robin; **Hirst, Timothy R.**
CS Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK
SO J. Gen. Microbiol. (1992), 138(4), 719-24
CODEN: JGMIAN; ISSN: 0022-1287
DT Journal
LA English
AB A marine Vibrio (designated Vibrio sp. 60) that is related to V. anguillarum was used as a host for a plasmid that encodes the non-toxic B subunit (EtxB) of E. coli heat-labile enterotoxin. Expression of EtxB in Vibrio sp. 60 resulted in the efficient and selective secretion of the B subunit into the extracellular growth medium. This indicated that Vibrio sp. 60, which does not normally produce cholera-like enterotoxins, nonetheless possesses a secretory machinery that permits these toxins to be translocated across its cytoplasmic and outer membranes. Expression of EtxB in a sec mutant of Vibrio sp. 60 (MVT1192), which had previously been shown to be defective in the secretion of several extracellular proteins, resulted in approx. 95% of the B subunit remaining entrapped within the periplasm of the bacterial cell envelope. This implies that the mutation in MVT1192 defines a locus that dets. a common step in the secretion of extracellular proteins, including oligomeric toxins.

L8 ANSWER 51 OF 67 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 36
AN 1993:89395 BIOSIS
DN PREV199344043645
TI Escherichia coli heat-labile enterotoxin B subunit as a carrier for delivery of a peptide with anti-HSV activity.
AU Marcello, Alessandro (1); Palu, Giorgio; **Hirst, Timothy R. (1)**
CS (1) Biol. Lab., Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO Biochemical Society Transactions, (1992) Vol. 20, No. 4, pp. 311S.
Meeting Info.: 643rd Meeting of the Biochemical Society Coventry, England, UK July 22-23, 1992
ISSN: 0300-5127.
DT Article

LA English

L8 ANSWER 52 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1992:102389 CAPLUS
DN 116:102389
TI Assembly and secretion of oligomeric toxins
AU **Hirst, Timothy R.**; Sandkvist, Maria; Aitken, Robert; Bagdasarian, Michael
CS Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK
SO FEMS Symp. (1991), 51(Microb. Surf. Compon. Toxins Relat. Pathog.), 181-90
CODEN: FEMSDW; ISSN: 0163-9188
DT Journal
LA English
AB This paper describes the mechanisms of protein export and secretion in bacterial cells, and in particular the secretion of complex oligomeric toxins that are responsible for causing cholera and related diarrheal diseases.

L8 ANSWER 53 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1992:608498 CAPLUS
DN 117:208498
TI Assembly and secretion of oligomeric toxins by Gram-negative bacteria
AU **Hirst, Timothy R.**
CS Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK
SO Sourceb. Bact. Protein Toxins (1991), 75-100. Editor(s): Alouf, Joseph E.; Freer, John H. Publisher: Academic, London, UK.
CODEN: 57WAAK
DT Conference; General Review
LA English
AB A review with 162 refs. Protein export and secretion in gram-neg. bacteria is discused.

L8 ANSWER 54 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1990:586381 CAPLUS
DN 113:186381
TI Minimal deletion of amino acids from the carboxyl terminus of the B subunit of heat-labile enterotoxin causes defects in its assembly and release from the cytoplasmic membrane of Escherichia coli
AU Sandkvist, Maria; **Hirst, Timothy R.**; Bagdasarian, Michael
CS Michigan Biotechnol. Inst., Michigan State Univ., Lansing, MI, 48909, USA
SO J. Biol. Chem. (1990), 265(25), 15239-44
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Minimal alterations at the carboxyl terminus of the B subunit (EtxB) of heat-labile enterotoxin from E. coli had a marked effect on the assembly and release of this polypeptide into the periplasm. Nine mutant EtxB polypeptides were obtained by genetic manipulation of the 3'-end of the etxB gene using Bal31 nuclease digestion and codon substitution. A correlation was obsd. between the magnitude of the changes introduced at the carboxyl terminus and the extent to which the mutant polypeptides were defective in assembly and release. Some of the mutant B subunits, exemplified by those in which the last 2 amino acids had been deleted or in which the last 4 residues had been replaced by three different ones, were only partially defective, with a proportion being assocd. with the periplasmic face of the cytoplasmic membrane and the remainder being exported to the periplasm. The portion assocd. with membranes was detected as monomers on sodium dodecyl sulfate-polyacrylamide gels, whereas the portion exported to the periplasm were detected as assembled oligomers. Apparently, the last few amino acids at the carboxyl terminus of EtxB exert a profound influence on the assembly and release of the B subunit from the cytoplasmic membrane during export in E. coli.

L8 ANSWER 55 OF 67 CAPLUS COPYRIGHT 2002 ACS

AN 1988:626424 CAPLUS
DN 109:226424
TI Coordinated assembly of multisubunit proteins: oligomerization of bacterial enterotoxins in vivo and in vitro
AU Hardy, Simon J. S.; Holmgren, Jan; Johansson, Susanne; Sanchez, Joaquin; **Hirst, Timothy R.**
CS Dep. Biol., Univ. York, York, YO1 5DD, UK
SO Proc. Natl. Acad. Sci. U. S. A. (1988), 85(19), 7109-13
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The assembly, in vivo and in vitro, of a family of hexameric, heat-labile enterotoxins produced by diarrheagenic bacteria was studied. The toxins, which consist of an A subunit and 5 B subunits, are assembled by a highly coordinated process that ensures secretion of the holotoxin complex. It was shown that (1) oxidn. of cysteine residues in the B subunits is a prerequisite step in vivo formation of B-subunit pentamers, (2) redn. of dissocd. B subunits in vitro abolishes their ability to reassemble, (3) the kinetics of B-pentamer assembly in vivo can be mimicked under defined conditions in vitro, (4) A subunits cannot assoc. with fully assembled B pentamers in vitro, and (5) A subunits cause an .apprx.3-fold acceleration in the rate of B-subunit pentamerization in vivo, implying that A subunits play a coordinating role in the pathway of holotoxin assembly. The last finding is likely to be of general significance, since it provides a mechanism for preferentially excluding or favoring certain intermediates in the assembly of multisubunit proteins.

L8 ANSWER 56 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1988:449312 CAPLUS
DN 109:49312
TI Hybrid enterotoxin LTA::STa proteins and their protection from degradation by in vivo association with B-subunits of Escherichia coli heat-labile enterotoxin
AU Sanchez, Joaquin; **Hirst, Timothy R.**; Uhlin, Bernt E.
CS Dep. Med. Microbiol., Univ. Goeteborg, Goeteborg, S413-46, Swed.
SO Gene (1988), 64(2), 265-75
CODEN: GENED6; ISSN: 0378-1119
DT Journal
LA English
AB Chimeric proteins exhibiting antigenic determinants of the heat-labile enterotoxin (LT) and heat-stable (STa) enterotoxins on the same mol. may provide a means to obtain immunoprophylactic and diagnostic reagents for Escherichia coli-caused diarrhea. Fusion of 2 different lengths of the STa gene to the C end of the A-subunit of LT (LTA) previously resulted in LTA::STa fusion proteins as monitored by GM1-ELISA. The approx. mol. size of the LTA::STa fusion proteins was detd. and further evidence of their hybrid nature was demonstrated by immunoblot anal. In order to obtain detectable amts. of these recombinant proteins it was essential to coexpress them with the resp. B-subunit of LT (LTB). This dependence on coexpression reflects the assocn. between the LTA::STa hybrids and LTB subunits. The resulting LTA::STa/LTB complexes were found in the E. coli periplasm, indicating that the exported hybrids, once assocd. with LTB, were stabilized and formed mols. that behaved essentially as native LT. The protective effect exerted by the B-subunit might conceivably be extended to other LTA-derived hybrid proteins, thus allowing the fusion of other foreign peptides to LTA and their subsequent recovery in the same fashion.

L8 ANSWER 57 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1988:525482 CAPLUS
DN 109:125482
TI Mechanisms for secretion of extracellular proteins by gram-negative bacteria
AU **Hirst, Timothy R.**; Welch, Rodney A.

CS Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK
SO Trends Biochem. Sci. (Pers. Ed.) (1988), 13(7), 265-9
CODEN: TBSCDB; ISSN: 0376-5067
DT Journal; General Review
LA English
AB A review with 38 refs. on an array of translocating systems of gram-neg. bacteria exclusively involved in the secretion of extracellular proteins.

L8 ANSWER 58 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1988:3221 CAPLUS
DN 108:3221
TI Conformation of protein secreted across bacterial outer membranes: a study of enterotoxin translocation from Vibrio cholerae
AU **Hirst, Timothy R.**; Holmgren, Jan
CS Dep. Genet., Univ. Leicester, Leicester, LE1 7RH, UK
SO Proc. Natl. Acad. Sci. U. S. A. (1987), 84(21), 7418-22
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The secretion of enterotoxin by V. cholerae is punctuated by the transient entry of the toxin subunits into the periplasm. The subunits oligomerize into an assembled holotoxin within the periplasm prior to their secretion across the outer membrane. The rate of toxin assembly was studied by pulse-labeling cells with [35S]-methionine and then monitoring the turnover of radiolabeled subunits as they assembled within the periplasm. The subunits entered the periplasm as monomers and assembled into oligomers with a half-time of .apprx.1 min. Since assembly was a rapid event compared to the rate of toxin efflux from the periplasm, which has a half-time of .apprx.13 min, it was concluded that all of the subunits that pass through the periplasm assemble before they traverse the outer membrane. The av. concn. of subunit monomers and assembly holotoxin within the periplasm was calcd. to be .apprx.20 and .apprx.260 .mu.g/mL, resp. This indicates that the periplasm is a suitably concd. milieu where spontaneous toxin assembly can occur. The findings suggest that protein movement across bacterial outer membranes, in apparent contrast to export across other biol. membranes, involves translocation of polypeptides that have already folded into tertiary and even quaternary conformations.

L8 ANSWER 59 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1987:594705 CAPLUS
DN 107:194705
TI Alterations at the carboxyl terminus change assembly and secretion properties of the B subunit of Escherichia coli heat-labile enterotoxin
AU Sandkvist, Maria; **Hirst, Timothy R.**; Bagdasarian, Michael
CS Inst. Appl. Cell Mol. Biol., Umea Univ., Umea, S-901 87, Swed.
SO J. Bacteriol. (1987), 169(10), 4570-6
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB The gene encoding the B subunit of heat-labile enterotoxin (etxB) was mutated at its 3' end by targeted addn. of random nucleotide sequences. Gene products from five mutated extB genes, all of which were shown to encode B subunits with short carboxy-terminal amino acid extensions, were analyzed with respect to a range of functional and structural properties. One class of altered B subunits, exemplified by EtxB124 and EtxB138, which both have extra amino acid residues, were found to be specifically defective in their ability to stably assoc. with A subunits and form holotoxin. Other altered B subunits were less subtly affected by extensions at their C termini and were, in addn. to their failure to assoc. with A subunits, unable to translocate into the periplasm of E. coli, to pentamerize, or to bind to GM1 ganglioside. This suggests that the carboxy-terminal domain of EtxB mediates A subunit-B subunit interaction.

L8 ANSWER 60 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1987:152759 CAPLUS
DN 106:152759
TI Transient entry of enterotoxin subunits into the periplasm occurs during their secretion from Vibrio cholerae
AU **Hirst, Timothy R.**; Holmgren, Jan
CS Dep. Med. Microbiol., Univ. Goeteborg, Goeteborg, S-41346, Swed.
SO J. Bacteriol. (1987), 169(3), 1037-45
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Cholera toxin and heat-labile enterotoxin (LT) are structurally similar oligomeric proteins which are capable of being efficiently secreted from V. cholerae. These proteins transiently enter the periplasm of V. cholerae as they traverse the cell envelope to reach the extracellular milieu. Pulse-chase expts. on V. cholerae TRH7000 harboring an LT-encoding plasmid revealed that radiolabeled LT A and B subunits entered the periplasm rapidly, followed by their slow efflux (half-time, 13 min) into the medium. LT B-subunit efflux from the periplasm was calcd. to be at a rate of .apprx.170 monomers/min-cell (which is equiv. to 34 assembled LT holotoxin mols./min-cell). These values were estd. to be sufficient to account for the increase in extracellular enterotoxin concn. during exponential cell growth. Thus, all enterotoxin subunits which are secreted into the medium can be assumed to be channelled via the periplasm. These findings led to an improved model of the pathway of toxin secretion by V. cholerae.

L8 ANSWER 61 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1986:566245 CAPLUS
DN 105:166245
TI Expression of the Escherichia coli lamB gene in Vibrio cholerae
AU Harkki, Anu; **Hirst, Timothy R.**; Holmgren, Jan; Palva, E. Tapio
CS Dep. Genet., Univ. Helsinki, Helsinki, SF-00100, Finland
SO Microb. Pathog. (1986), 1(3), 283-8
CODEN: MIPAEV; ISSN: 0882-4010
DT Journal
LA English
AB A phage .lambda.-mediated transduction system was devised to facilitate mol. anal. of V. cholerae. A lamB expression plasmid pAMH62 was introduced into V. cholerae by conjugation. The resulting V. cholerae derivs. harboring pAMH62 produced substantial amts. of the LamB protein. This protein was poorly inserted into the outer membrane, as suggested by its location in the cell envelope, its assocn. with the peptidoglycan layer of the cell wall, and its function as receptor for phage .lambda.. In vivo packaged cosmids were efficiently transduced into these strains of V. cholerae.

L8 ANSWER 62 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1985:92697 CAPLUS
DN 102:92697
TI Mechanism of toxin secretion by Vibrio cholerae investigated in strains harboring plasmids that encode heat-labile enterotoxins of Escherichia coli
AU **Hirst, Timothy R.**; Sanchez, Joaquin; Kaper, James B.; Hardy, Simon J. S.; Holmgren, Jan
CS Dep. Med. Microbiol., Univ. Goeteborg, Goeteborg, S-413 46, Swed.
SO Proc. Natl. Acad. Sci. U. S. A. (1984), 81(24), 7752-6
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB A genetically engineered V. cholerae strain from which the cholera toxin genes had previously been deleted was used as a host in which to study the expression and secretion of related toxins and their subunits. Recombinant plasmids encoding heat-labile enterotoxins (LTs) from E. coli

of human and porcine origin were expressed in the V. cholerae host, and this resulted in the secretion of the LTs into the extracellular milieu. The A subunits of human and porcine LT were unnicked polypeptides, which indicates that nicking is not obligatory for toxin secretion. V. cholerae Strains were also constructed that harbored plasmids encoding either the A or the B subunits of human LT (A+B-, or A-B+). Approx. 90% of the B subunits were secreted from the A-B+ strain, whereas all of the A subunits expressed by the A+B- strain remained cell assocd. Apparently, strains that synthesize both subunits assemble the A and B subunits prior to their secretion. The entry of the toxin into the secretory step of the export pathway apparently is mediated by a secretory app. that recognizes structural domains within the B subunit of LT.

L8 ANSWER 63 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1984:117539 CAPLUS
DN 100:117539
TI Cellular location of heat-labile enterotoxin in Escherichia coli
AU **Hirst, Timothy R.**; Randall, Linda L.; Hardy, Simon J. S.
CS Dep. Biol., Univ. York, Heslington/York, Y01 5DD, UK
SO J. Bacteriol. (1984), 157(2), 637-42
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB Both the A and B subunits of heat-labile enterotoxin from E. coli are located in the periplasm. The toxin was shown to form aggregates in Tris-EDTA buffers which are routinely used for isolating membranes. The aggregates pellet upon centrifugation, and this may explain why several previous investigators have concluded that enterotoxin is assocd. with membranes.

L8 ANSWER 64 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1984:188499 CAPLUS
DN 100:188499
TI Cellular location of enterotoxin in Escherichia coli
AU **Hirst, Timothy R.**; Randall, Linda L.; Hardy, Simon J. S.
CS Dep. Biol., Univ. York, York, YO1 5DD, UK
SO Biochem. Soc. Trans. (1984), 12(2), 189-91
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English
AB In E. coli, enterotoxin is a sol. protein located in the periplasmic space of the cell envelope. In buffers of low ionic strength, enterotoxin forms aggregates which then pellet with spheroplasts. To understand the pathogenesis of enterotoxinogenic E. coli, it will be necessary to describe the release or export of enterotoxin from its periplasmic pool.

L8 ANSWER 65 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1983:68624 CAPLUS
DN 98:68624
TI Assembly in vivo of enterotoxin from Escherichia coli: formation of the B subunit oligomer
AU **Hirst, Timothy R.**; Hardy, Simon J. S.; Randall, Linda L.
CS Biochem. Biophys. Program, Washington State Univ., Pullman, WA, 99164-4630, USA
SO J. Bacteriol. (1983), 153(1), 21-6
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB An oligomer of the B subunit of heat-labile enterotoxin of E. coli was obsd. in minicells and in whole cells. There is a delay after synthesis of the B subunit before it appears in the oligomer. The delay is not due to slow processing of the precursor. A similar delay in oligomerization of the major outer membrane protein OmpF is also described.

L8 ANSWER 66 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1981:188325 CAPLUS
DN 94:188325
TI Synthesis of a precursor to the B subunit of heat-labile enterotoxin in Escherichia coli
AU Palva, E. Tapio; **Hirst, Timothy R.**; Hardy, Simon J. S.; Holmgren, Jan; Randall, Linda
CS Mol. Biol. Inst., Uppsala Univ., Uppsala, S-751 22, Swed.
SO J. Bacteriol. (1981), 146(1), 325-30
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
AB E. coli K-12 minicells were used to investigate the synthesis of the plasmid-coded, heat-labile enterotoxin of E. coli. Two polypeptides related to the B subunit of the toxin were expressed in the minicells. One of these polypeptides (mol. wt. 11,500) was immunopptd. by antiserum to cholera toxin. Because the B subunits of heat-labile enterotoxin and cholera toxin have common antigenic sites, this species was probably the mature B subunit. The larger polypeptide (mol. wt. 13,000) was likely a precursor of the B subunit, because it could be chased into the mature form. This conversion was inhibited by compds. which dissipated proton motive force, suggesting that the processing requires energy.

L8 ANSWER 67 OF 67 CAPLUS COPYRIGHT 2002 ACS
AN 1981:403206 CAPLUS
DN 95:3206
TI Energy is required for maturation of exported proteins in Escherichia coli
AU Enequist, Hans G.; **Hirst, Timothy R.**; Harayama, Shigeaki; Hardy, Simon J. S.; Randall, Linda L.
CS Wallenberg Lab., Uppsala Univ., Uppsala, Swed.
SO Eur. J. Biochem. (1981), 116(2), 227-33
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB An energy requirement for the proteolytic processing of 5 exported proteins (gene ompF, gene ompA, gene lamB, maltose-binding, and arabinose-binding proteins) of E. coli is reported. Studies with an uncA mutant suggest that the form of energy required is protonmotive force. Thus, an energized membrane is probably essential for export of periplasmic and outer-membrane proteins.

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L6 ANSWER 1 OF 34 USPATFULL
AN 2001:159338 USPATFULL
TI Digital X-ray imaging system with automatic display image greyscale enhancement and method
IN **Williams, Neil A.**, Los Gatos, CA, United States
May, Gerald A., Saratoga, CA, United States
PA Canon Kabushiki Kaisha, Tokyo, Japan (non-U.S. corporation)
PI US 6292535 B1 20010918
AI US 1999-468744 19991221 (9)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Kim, Robert H.; Assistant Examiner: Thomas, Courtney
LREP Hentzel, Paul
CLMN Number of Claims: 32
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 867
AB Digital X-ray imaging system 10 automatically provides an enhanced digital display image 10D from a digital camera image 10C showing internal structure 10S of interest within subject 11S. Calibrated radiation attenuators 10A placed near the subject appear in the camera image and in the display image. X-ray source 10X generates X-ray radiations which are attenuated within the calibrated attenuators to provide calibrated attenuations. The X-ray radiations are also differentially attenuated within the interior of the subject to reveal internal structure therein. Digital X-ray camera 11C detects the calibrated radiations to form the camera image of the calibrated greyscale levels. The camera also detects the differentially attenuated radiations to provide the camera image of internal structure of the subject. Each calibrated attenuator appears in the camera image as a collection of adjacent camera pixels exhibiting one of the plurality of calibrated greyscale levels within the camera greyscale. Retriever 12 retrieves the calibrated greyscale level from the digital camera image of each attenuator pixel collection. Greyscale coordinator 14 is responsive to the retrieved calibrated greyscale levels for determining mapping assignments defining the relationship between the camera greyscale and the display greyscale which provides the enhanced digital display image. Greyscale mapper 11M is responsive to the mapping assignments for mapping the camera greyscale levels of the camera pixels forming the camera image into display greyscale levels of display pixels forming the enhanced display image. Display monitor 11D is responsive to the enhanced display image for displaying a pixel image of the internal structure of interest within the subject.

L6 ANSWER 2 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2001:397621 BIOSIS
DN PREV200100397621
TI Escherichia coli enterotoxin B subunit triggers apoptosis of CD8+ T cells by activating transcription factor c-Myc.
AU Soriani, Marco; **Williams, Neil A.**; Hirst, Timothy R. (1)
CS (1) Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD: t.r.hirst@bristol.ac.uk UK
SO Infection and Immunity, (August, 2001) Vol. 69, No. 8, pp. 4923-4930. print.
ISSN: 0019-9567.
DT Article
LA English
SL English
AB Heat-labile enterotoxin from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile enterotoxin (EtxB) was found to induce the selective death of CD8+ T cells, suggesting that EtxB may

trigger activation of proapoptotic signaling pathways. Here we show that EtxB treatment of CD8+ T cells but not of CD4+ T cells triggers the specific up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for EtxB-mediated apoptosis. Myc activation was also associated with an increase of IkappaBalpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8+ T cells were pretreated with inhibitors of IkappaBalpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both EtxB-induced apoptosis and Myc expression. Further, a non-receptor-binding mutant of EtxB, EtxB(G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that EtxB is a signaling molecule that triggers activation of transcription factors involved in cell survival.

L6 ANSWER 3 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2001:229251 CAPLUS
DN 135:26350
TI A Reflection-Absorption Infrared Spectroscopy (RAIRS) Investigation of the Low-Temperature Heterogeneous Hydrolysis of Bromine Nitrate
AU Gane, Matt P.; **Williams, Neil A.**; Sodeau, John R.
CS Department of Chemistry, University College, Cork, Ire.
SO Journal of Physical Chemistry A (2001), 105(16), 4002-4009
CODEN: JPCAFH; ISSN: 1089-5639
PB American Chemical Society
DT Journal
LA English
AB Reflection-absorption IR spectroscopy (RAIRS) was employed to study the heterogeneous hydrolysis of Br nitrate (BrONO2) in and on thin ice films between 95 and 185 K. Hydrolysis occurs throughout the temp. range with evidence for 2 competing mechanisms. At the stratospherically relevant temp. of 185 K, the reaction proceeds by an ionic mechanism to give the hypobromous ion, H2OBr+, and both HNO3 di- and trihydrate. A mol. mechanism is also operative to give hypobromous acid, HOBr, and hydrated HNO3 (HNO3.cntdot.(H2O)n). This 2nd route becomes more important under conditions of limited H2O presence and at lower temps. All of the results are compared to analogous studies previously performed on Cl nitrate (ClONO2).
RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:71954 BIOSIS
DN PREV200200071954
TI MHC matching and mechanisms of alloactivation in corneal transplantation.
AU Nicholls, Susan M. (1); **Williams, Neil A.**
CS (1) Division of Ophthalmology, School of Medical Sciences, University Walk, Bristol, BS8 1TD UK
SO Transplantation (Baltimore), (November 15, 2001) Vol. 72, No. 9, pp. 1491-1497. print.
ISSN: 0041-1337.
DT Article
LA English
AB Background: In human corneal transplantation the value of matching, particularly for MHC class II, is unclear and controversial. The contribution of the direct pathway to T cell activation is also uncertain. We have determined the relative contribution of class I, II and non-MHC antigens to graft rejection and of the direct and indirect pathways to T cell activation in a rat model mimicking human incompatibilities. Methods: DA (RT1a) strain recipients received fully mismatched PVG (RT1c) strain grafts or grafts from one of three recombinant strains bearing DA MHC genes on a PVG background. Graft survival was assessed and the specificity

of T cells generated in the draining lymph nodes was determined in mixed lymphocyte (MLR) proliferation assays. To assess the contribution of the direct pathway, fully mismatched graft were performed and allospecific proliferation was measured after depletion of recipient APC from the MLR reaction. Results: There was no significant difference in survival of grafts between the four grades of mismatch, which ranged from a full mismatch to non-MHC mismatches alone (median survival 12.5, 11, 13 and 12.5 days respectively). In conformity with clinical results, strong secondary responses were generated against targets matched for MHC with the recipient. Depletion of recipient APC from a fully allogeneic secondary MLR did not fully abrogate donor-specific proliferation. Conclusions: Class II matching is of no benefit in this model. Strong indirect responses to non-MHC mismatches are sufficient to induce the rapid rejection, but the small numbers of class II+ cells in the donor appear sufficient to generate a direct response.

L6 ANSWER 5 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 2001:325450 BIOSIS
DN PREV200100325450
TI Antigen-receptor cross-linking and lipopolysaccharide trigger distinct phosphoinositide 3-kinase-dependent pathways to NF-kappaB activation in primary B cells.
AU Bone, Heather; **Williams, Neil A. (1)**
CS (1) Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD UK
SO International Immunology, (June, 2001) Vol. 13, No. 6, pp. 807-816. print. ISSN: 0953-8178.
DT Article
LA English
SL English
AB The NF-kappaB/Rel transcription factors play an important role in the expression of genes involved in B cell development, differentiation and function. Nuclear NF-kappaB is induced in B cells by engagement of either the BCR or CD40 or by stimulation with lipopolysaccharide (LPS). Despite the importance of NF-kappaB to B cell function, little is known about the signaling pathways leading to NF-kappaB activation. In this report we address the role of phosphoinositide 3'-kinase (PI 3-kinase) in BCR- and LPS-induced NF-kappaB activation using populations of primary murine resting B cells. Using the specific pharmacological inhibitors of PI 3-kinase, Wortmannin and LY294002, we demonstrate that PI 3-kinase activity is vital for BCR-induced NF-kappaB DNA-binding activity. Furthermore, we show that this is achieved via protein kinase C-dependent degradation of IkappaBalpha. Similar analyses reveal that PI 3-kinase is also critical in triggering NF-kappaB DNA-binding activity and IkappaBalpha degradation following LPS stimulation. Interestingly, a PKC inhibitor which blocked the BCR-induced IkappaBalpha degradation had no effect on the degradation of IkappaBalpha after LPS stimulation. Taken together, our results indicate the involvement of PI 3-kinase in at least two distinct signaling pathways leading to activation of NF-kappaB in B cells.

L6 ANSWER 6 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 2001:429053 BIOSIS
DN PREV200100429053
TI The genetic and immunopathological processes underlying collagen-induced arthritis.
AU Luross, Jeff A.; **Williams, Neil A. (1)**
CS (1) Department of Pathology and Microbiology, University of Bristol, University Walk, Bristol, BS8 1TD: neil.a.williams@bris.ac.uk UK
SO Immunology, (August, 2001) Vol. 103, No. 4, pp. 407-416. print. ISSN: 0019-2805.
DT General Review

LA English
SL English
AB Animal models of rheumatoid arthritis (RA) have provided substantial insights into basic pathogenic mechanisms of chronic inflammatory arthritis and autoimmune disease in general. Of the variety of models reported, collagen-induced arthritis (CIA) has been the most characterized in terms of both its pathogenesis and its underlying immunological basis. Collagen-induced arthritis has also been the model of choice in terms of testing potential new therapeutic agents for the treatment of human RA. Nevertheless, the complex nature of the balance between T-cell cytokines and the chronic inflammatory processes is only recently becoming clear. This review focuses on these developments, highlighting their implications for our understanding of RA and for the use of CIA as a suitable animal model.

L6 ANSWER 7 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:152196 BIOSIS
DN PREV200100152196
TI Cell identification and isolation on the basis of cytokine secretion: A novel tool for investigating immune responses.
AU Turcanu, Victor; **Williams, Neil A. (1)**
CS (1) Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol: neil.a.williams@bristol.ac.uk UK
SO Nature Medicine, (March, 2001) Vol. 7, No. 3, pp. 373-376. print.
ISSN: 1078-8956.
DT Article
LA English
SL English

L6 ANSWER 8 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:220859 BIOSIS
DN PREV200100220859
TI Immunomodulation of the human MLR by E. coli - heat-labile toxin B subunit (EtxB): Induction of regulatory T cells.
AU **Williams, Neil A. (1)**; Turcanu, Victor (1)
CS (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 ITD UK
SO Diabetes-Metabolism Research and Reviews, (January February, 2001) Vol. 17, No. Suppl. 1, pp. S37. print.
Meeting Info.: 5th International Congress of the Immunology of Diabetes Society Madras, Chennai, India February 13-16, 2001
ISSN: 1520-7552.
DT Conference
LA English
SL English

L6 ANSWER 9 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:220695 BIOSIS
DN PREV200100220695
TI Nasal administration of admixed E. coli heat-labile toxin B subunit (EtxB) and insulin prevents autoimmune diabetes melitus (iDDM) in NOD mice by inducing regulatory CD4+ cells.
AU **Williams, Neil A. (1)**; Turcanu, Victor (1)
CS (1) Department of Pathology and Microbiology, University of Bristol, University Walk, Bristol, BS8 1TD UK
SO Diabetes-Metabolism Research and Reviews, (January February, 2001) Vol. 17, No. Suppl. 1, pp. S36. print.
Meeting Info.: 5th International Congress of the Immunology of Diabetes Society Madras, Chennai, India February 13-16, 2001
ISSN: 1520-7552.
DT Conference
LA English

SL English

L6 ANSWER 10 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2000:303839 CAPLUS
DN 132:353149
TI An ab initio and experimental study of bromine on low-temperature water clusters and ice surfaces
AU Ramondo, Fabio; Sodeau, John R.; Roddis, Tristan B.; **Williams, Neil A.**
CS Department of Chemistry, Chem. Eng. Mater., University of L'Aquila, L'Aquila, Italy
SO Phys. Chem. Chem. Phys. (2000), 2(10), 2309-2318
CODEN: PPCPFQ; ISSN: 1463-9076
PB Royal Society of Chemistry
DT Journal
LA English
AB A dual RAIR (reflection-absorption IR) spectroscopy and ab initio study of the interaction between Br2 and water-ice was performed. The spectra were measured in the temp. range 85-180 K and the results compared to theor. models of Br-H2O clusters and hydrated H2OX+ and X2OH- ions. Particular theor. attention was paid to the possibility that the interacting mols. modify their properties (geometry, partial at. charge and vibrational frequencies) with the size of the water clusters i.e. Br2.cntdot..cntdot..cntdot.(H2O)n. Addnl., ab initio methods were employed to investigate the actual mechanism of the Br2 hydrolysis by evaluating the stability of possible reaction intermediates such as [H2OBr]+ in the gas-phase as well as upon solvation. The chem. structures and vibrational frequencies of the hydrated ion-pairs were also examd. The theor. treatments employed show that the Br2 and water mols. can form a weakly bonded binary complex with interaction between the Br- and O-atoms. Furthermore, the exptl. evidence obtained for Br2 hydrolysis occurring via an [H2OBr]+ ion on water-ice is investigated by the energy calcns., which show that the route is competitive with that occurring via an H3O+ ion. The vibrational frequencies calcd. for a hydrated H2OBr+ Br- ion pair satisfactorily reproduce the set of RAIR spectroscopy expts. preformed at 180 K.
RE.CNT 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:82983 BIOSIS
DN PREV200100082983
TI Nasal administration of admixed E. coli heat-labile toxin B subunit (EtxB) and insulin prevents autoimmune diabetes mellitus (IDDM) in NOD mice by inducing regulatory CD4+ cells.
AU Turcanu, Victor (1); **Williams, Neil A. (1)**
CS (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK
SO Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print.
Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08, 2000 British Society for Immunology
. ISSN: 0019-2805.
DT Conference
LA English
SL English

L6 ANSWER 12 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:82982 BIOSIS
DN PREV200100082982
TI Immunomodulation of the human MLR by E. coli-heat-labile toxin B subunit (EtxB): Induction of regulatory T cells.
AU Turcanu, Victor (1); **Williams, Neil A. (1)**
CS (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK

SO Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 59. print. Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08,. 2000 British Society for Immunology . ISSN: 0019-2805.
DT Conference
LA English
SL English

L6 ANSWER 13 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:82879 BIOSIS
DN PREV200100082879
TI Modulation of B cell signalling events induced by the B-subunit of E. coli heat labile enterotoxin.
AU Bone, Heather K. (1); Jackson, Michelle E. (1); **Williams, Neil A.** **(1)**
CS (1) Dept. of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK
SO Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 23. print. Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08, 2000 British Society for Immunology . ISSN: 0019-2805.
DT Conference
LA English
SL English

L6 ANSWER 14 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 2000:883742 CAPLUS
DN 135:44842
TI Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic
AU Pitman, Richard S.; Hirst, Timothy R.; **Williams, Neil A.**
CS Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA
SO Recent Research Developments in Immunology (1999), 1(Pt. 2), 497-511 CODEN: RRDIB8
PB Research Signpost
DT Journal; General Review
LA English
AB A review with 59 refs. Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation, and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.
RE.CNT 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 15 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:155508 BIOSIS
DN PREV200000155508
TI Signalling events induced by E. coli heat-labile enterotoxin B-subunit-receptor binding.
AU Bone, Heather K. (1); Pitman, Richard S. (1); **Williams, Neil A.** **(1)**
CS (1) Dept. Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK

SO Immunology., (Dec., 1999) Vol. 98, No. suppl. 1, pp. 174.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy & Clinical Immunology. Harrogate, England, UK November 30-December 03, 1999 British Society for Allergy & Clinical Immunology
. ISSN: 0019-2805.
DT Conference
LA English
SL English

L6 ANSWER 16 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 1999:459318 BIOSIS
DN PREV199900459318
TI Immune modulation by the cholera-like enterotoxins: From adjuvant to therapeutic.
AU **Williams, Neil A. (1)**; Hirst, Timothy R. (1); Nashar, Toufic O. (1)
CS (1) Dept of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD UK
SO Immunology Today, (Feb., 1999) Vol. 20, No. 2, pp. 95-101.
ISSN: 0167-5699.
DT General Review
LA English

L6 ANSWER 17 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:155165 BIOSIS
DN PREV200000155165
TI Induction of immune regulation by the cholera-like enterotoxins.
AU **Williams, Neil A. (1)**
CS (1) Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD UK
SO Immunology., (Dec., 1999) Vol. 98, No. suppl. 1, pp. 7.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy and Clinical Immunology. Harrogate, England, UK November 30-December 03, 1999 British Society for Allergy and Clinical Immunology
. ISSN: 0019-2805.
DT Conference
LA English
SL English

L6 ANSWER 18 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1998:424983 CAPLUS
DN 129:205411
TI Mechanism of the heterogeneous reaction of hydrogen chloride with chlorine nitrate and hypochlorous acid on water ice
AU Horn, Andrew B.; Sodeau, John R.; Roddis, Tristan B.; **Williams, Neil A.**
CS Department of Chemistry, University of York, Heslington York, YO1 5DD, UK
SO J. Phys. Chem. A (1998), 102(30), 6107-6120
CODEN: JPCAFH; ISSN: 1089-5639
PB American Chemical Society
DT Journal
LA English
AB The interactions of HOCl and ClONO2 with pure and HCl-doped water ice have been reinvestigated using IR spectroscopy in conjunction with static and thermal desorption mass spectrometry in the temp. range 140-180 K to probe the detailed mechanisms of their heterogeneous atm. reactions. In agreement with earlier studies, HOCl was found to react with hydroxonium chloride species to directly produce mol. chlorine. This mol. chlorine desorbed from the surface above ca. 155 K either directly or in subsequent thermal desorption expts., depending upon substrate temp. at the time of reaction. ClONO2 was obsd. to heterogeneously hydrolyze upon water ice to

produce HOCl and hydrated H3O+NO3-. Below ca. 155 K, HOCl remains adsorbed on the ice surface, while above ca. 155 K, it desorbs directly into the gas phase. These results suggest that a long-lived, adsorbed state of HOCl is unlikely to play a direct role in heterogeneous chem. at stratospheric temps. The direct reaction of ClONO2 with an ice surface satd. with ionic hydrates of HCl was obsd. to result in the prodn. of mol. chlorine, which, as for the HOCl reaction, either remained adsorbed or desorbed from the surface depending upon temp. This provides convincing evidence for the direct heterogeneous reaction of Cl- with ClONO2 under stratospheric conditions. A novel explanation for the enhanced reactivity of ClONO2 toward atmospherically relevant substrates is presented, in which a partially ionized precursor state is formed. Partial ionization of ClONO2 along the Cl-O bond serves to increase the electrophilicity of the chlorine atom, making the site highly prone to nucleophilic attack by either water or adsorbed chloride ions.

L6 ANSWER 19 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1998:339910 CAPLUS
DN 129:138697
TI Low temperature reaction of chlorine nitrate with water ice. Formation of molecular nitric acid
AU Horn, Andrew B.; Sodeau, John R.; Roddis, Tristan B.; **Williams, Neil A.**
CS School of Chemical Sciences, University of East Anglia, Norwich, NR4 7TJ, UK
SO J. Chem. Soc., Faraday Trans. (1998), 94(12), 1721-1724
CODEN: JCFTEV; ISSN: 0956-5000
PB Royal Society of Chemistry
DT Journal
LA English
AB We have examd. the reaction of ClONO2 with water ice at 140 K and found evidence for the formation of mol. nitric acid under conditions of reduced surface water. This is very different from the behavior of ClONO2 on ice at 180 K, where substrate-induced pre-reaction ionization of ClONO2 leads to a reaction mechanism involving the intermediacy of [H2OCl]+ and nitrate ions. These two results are not inconsistent with a single mechanism if a subtle change in the interplay between the availability of water mols. at the surface and the variation of the substrate/adsorbate interactions of ice/ClONO2 with temp. are taken into account. This implies that reaction mechanisms and product branching ratios in the atm. may vary widely over a range of temps. and reactant partial pressures.

L6 ANSWER 20 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
AN 1998:434383 BIOSIS
DN PREV199800434383
TI CD4+ T cells inhibit growth of Epstein-Barr virus-transformed B cells through CD95-CD95 ligand-mediated apoptosis.
AU Wilson, A. Douglas (1); Redchenko, Irina; **Williams, Neil A.**; Morgan, Andrew J.
CS (1) Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK
SO International Immunology, (Aug., 1998) Vol. 10, No. 8, pp. 1149-1157.
ISSN: 0953-8178.
DT Article
LA English
AB Greater than 90% of the human population acquire Epstein-Barr virus (EBV) in infancy and retain a lifelong latent Infection without any clinical consequences. Nevertheless EBV has been identified as the causal agent of infectious mononucleosis, and is associated with several tumours including endemic Burkitt's lymphoma and B cell lymphomas in immunosupressed patients. B cells infected with EBV are transformed In vitro and grow continuously as lymphoblastoid cell lines. The growth of EBV-transformed B cells In vivo is controlled by the immune system. Studies on immunity to

EBV have mainly focused on MHC class I-restricted CD8+ cytotoxic T cells specific for viral latent antigens. Here it is reported that in vitro stimulation of peripheral blood lymphocytes by autologous EBV-infected B cells, which have been induced to express lytic cycle antigens, gives rise to a predominantly CD4+ T cell response. Furthermore, the growth of EBV-infected B cells can also be regulated by these activated CD4+ T cells through apoptosis mediated by CD95-CD95 ligand (CD95L). CD95-CD95L-mediated apoptosis is an important mechanism of normal B cell growth regulation. As EBV-transformed B cells remain susceptible to this mechanism, the control of EBV in vivo may be not only by virus-specific CD8+ cytotoxic T cell immunity but also by normal mechanisms of immune regulation of B cell growth.

L6 ANSWER 21 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1999:144553 BIOSIS
DN PREV199900144553
TI Receptor mediated apoptosis of CD89+T cells by the B subunits of cholera-like enterotoxins.
AU Pitman, Richard S.; Hirst, Timothy R.; Nashar, Toufic O.; **Williams, Neil A.**
CS Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK
SO Biochemical Society Transactions, (Nov., 1998) Vol. 26, No. 4, pp. S338. Meeting Info.: 666th Meeting of the Biochemical Society Sheffield, England, UK July 29-31, 1998
ISSN: 0300-5127.
DT Conference
LA English

L6 ANSWER 22 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 8
AN 1997:274651 BIOSIS
DN PREV199799566369
TI Prevention of autoimmune disease due to lymphocyte modulation by the B-subunit of Escherichia coli heat-labile enterotoxin.
AU **Williams, Neil A.**; Stasiuk, Lisa M.; Nashar, Toufic O.; Richards, Claire M.; Lang, Allison K.; Day, Michael J.; Hirst, Timothy R.
CS Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK
SO Proceedings of the National Academy of Sciences of the United States of America, (1997) Vol. 94, No. 10, pp. 5290-5295.
ISSN: 0027-8424.
DT Article
LA English
AB We demonstrate that the receptor binding moiety of Escherichia coli heat-labile enterotoxin (EtxB) can completely prevent autoimmune disease in a murine model of arthritis. Injection of male DBA/1 mice at the base of the tail with type II collagen in the presence of complete Freund's adjuvant normally leads to arthritis, as evidenced by inflammatory infiltration and swelling of the joints. A separate injection of EtxB at the same time as collagen challenge prevented leukocyte infiltration, synovial hyperplasia, and degeneration of the articular cartilage and reduced clinical symptoms of disease by 82%. The principle biological property of EtxB is its ability to bind to the ubiquitous cell surface receptor GM1 ganglioside, and to other galactose-containing glycolipids and galactoproteins. The importance of receptor interaction in mediating protection from arthritis was demonstrated by the failure of a non-receptor-binding mutant of EtxB to elicit any protective effect. Analysis of T cell responses to collagen, in cultures of draining lymph node cells, revealed that protection was associated with a marked increase in interleukin 4 production concomitant with a reduction in interferon gamma levels. Furthermore, in protected mice there was a significant reduction in anti-collagen antibody levels as well as an increase in the IgG1/IgG2a ratio. These observations show that protection is associated with a shift in the Th1/Th2 balance as well as a general reduction in the

extent of the anti-type II collagen immune response. This suggests that EtxB-receptor-mediated modulation of lymphocyte responses provides a means of preventing autoimmune disease.

L6 ANSWER 23 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 9
AN 1998:49516 BIOSIS
DN PREV199800049516
TI Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1.
AU Shimeld, Carolyn (1); Whiteland, Joanne L.; **Williams, Neil A.**; Easty, David L.; Hill, Terry J.
CS (1) Dep. Ophthalmol., Sch. Med. Sci., Bristol BS8 1TD UK
SO Journal of General Virology, (Dec., 1997) Vol. 78, No. 12, pp. 3317-3325. ISSN: 0022-1317.
DT Article
LA English
AB Immunocytochemistry on serial paraffin sections was used to monitor the production dynamics of cytokines (IL-2, IL-4, IL-6, IL-10, IFN-gamma and TNF-alpha) and viral antigens in the trigeminal ganglion (TG) and the central side of the dorsal root entry zone (DRE) of mice, following infection of the cornea with herpes simplex virus type 1. In normal TG, scattered satellite cells were TNF-alpha+ and in the DRE, TNF-alpha+ and/or low numbers of IL-6+ cells were detected. On day 3 after infection, foci of TG neurons with viral antigens were surrounded by large numbers of TNFalpha+ and/or IL-6+ cells and low numbers of IFN-gamma+ cells. IL-2+ and/or IL-4+ cells appeared later, when viral antigens had almost cleared. In the TG, the most striking changes occurred with TNF-alpha, with respect to its source (satellite cells, Schwann cells and infiltrating cells) and the extent and long duration of its production. TNF-alpha was the predominant cytokine throughout acute and latent infection and even by day 30, numbers of satellite cells expressing this cytokine were three times higher than those in normal ganglia. Moreover, in the DRE, TNF-alpha was the only cytokine detected during virus clearance and again, its production continued, along with that of IL-6, on days 20 to 30, in both infiltrating cells and astrocytes. Thus, cytokines, particularly TNF-alpha and perhaps IL-6, from infiltrating cells and resident glial cells may have a role both in virus clearance and in normal homeostatic mechanisms in the nervous system such as repair and protection of neurons from damage.

L6 ANSWER 24 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1997:579648 CAPLUS
DN 127:282479
TI Reflection-absorption IR spectroscopic investigation of the photolysis of thin films of dichlorine monoxide and chlorine dioxide
AU Gane, Matt P.; **Williams, Neil A.**; Sodeau, John R.
CS School of Chemical Sciences, University of East Anglia, Norwich, NR4 7TJ, UK
SO J. Chem. Soc., Faraday Trans. (1997), 93(16), 2747-2754
CODEN: JCFTEV; ISSN: 0956-5000
PB Royal Society of Chemistry
DT Journal
LA English
AB Reflection-absorption IR (RAIR) spectroscopy and mass spectrometry have been employed in order to investigate the low-temp. photochem. of thin films of chlorine dioxide, OClO, and dichlorine monoxide, Cl2O, grown on a gold foil in an ultra-high vacuum system. Photolysis of a neat film of OClO (.lambda. > 300 nm, 90-110 K) produces chloryl chloride, ClClO2. Irradn. of a co-deposited OClO/H2O film also produces chlorine superoxide, ClOO, which suggests that OClO isomerization is the first step in the reaction producing ClClO2. Photolysis of Cl2O (300 < .lambda. <515 nm, 90-110 K) is shown to produce OClO, initially, which is subsequently converted to ClClO2. Anal. of the obsd. IR band intensities and

consideration of the metal surface selection (MSS) rule indicates that the photochem. produced OClO intermediate aligns perpendicular to the gold substrate. Mechanistic details and the atm. implications of the chem. are discussed.

L6 ANSWER 25 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1997:19090 CAPLUS
TI Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: an in vivo study of virus antigen and immune cell infiltration
AU Shimeld, Carolyn; Whiteland, Joanne L.; **Williams, Neil A.**; Easty, David L.; Hill, Terry J.
CS UK
SO J. Gen. Virol. (1996), 77(12), 3165
CODEN: JGVIAY; ISSN: 0022-1317
PB Society for General Microbiology
DT Journal; Errata
LA English
AB Unavailable

L6 ANSWER 26 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 10
AN 1996:526169 BIOSIS
DN PREV199699248525
TI Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: An in vivo study of virus antigen and immune cell infiltration.
AU Shimeld, Carolyn (1); Whiteland, Joanne L.; **Williams, Neil A.**; Easty, David L.; Hill, Terry J.
CS (1) Dep. Ophthalmol., Sch. Med. Sci., Bristol BS8 1TD UK
SO Journal of General Virology, (1996) Vol. 77, No. 10, pp. 2583-2590. ISSN: 0022-1317.
DT Article
LA English
AB The corneas of latently infected mice were UV irradiated to induce reactivation of herpes simplex virus type 1 (HSV-1) in the trigeminal ganglion (TG). On days 1 to 4 after irradiation, TG were removed, serially sectioned and double stained to identify immune cells and virus antigens. Virus antigen was detected in small numbers (most commonly one) of neurons per ganglion as early as day 1, confirming the rapidity of reactivation and the neuron as the likely site of this event. The immune response was also rapid and effective since virus antigen was identified in immune cells at day 1 and by day 4 all samples were negative. The predominant infiltrating cells on days 1 and 2, when virus antigen was present and being cleared, were T cells, both CD4+ and CD8+. Later, large numbers of B cells appeared, suggesting that local antibody production may also be involved in controlling the reactivated infection. The observations suggest that a significant proportion of reactivation events do not result in disease of the eye or shedding of virus in the tear film. However, they also suggest that as little as one reactivating neuron in the ganglion may be sufficient to lead to such disease and/or shedding.

L6 ANSWER 27 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 11
AN 1996:317111 BIOSIS
DN PREV199699039467
TI Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes.
AU Nashar, Toufic O. (1); **Williams, Neil A.**; Hirst, Timothy R.
CS (1) Res. Sch. Biosciences, Univ. Kent, Canterbury, Kent CT2 7NJ UK
SO International Immunology, (1996) Vol. 8, No. 5, pp. 731-736. ISSN: 0953-8178.
DT Article
LA English
AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the

membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (EtxB) of Escherichia coli heat-labile enterotoxin (Etx) is a potent immunogen in vivo and has profound modulatory effects on EtxB-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by EtxB causes a differential effect on mature CD4+ and CD8+ T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of EtxB to such cultures led to the complete depletion of CD8+ T cells compared with enhanced activation of CD4+ T cells (as measured by expression of CD25 (IL-2R-alpha)). By contrast, addition of a mutant EtxB, EtxB(G33D), which does not bind to GM1, failed to trigger CD8+ T cell depletion. When EtxB was added to isolated non-immune CD8+ lymphocytes rapid, (12-18 h) alterations in nuclear morphology and the appearance of sub-G-0/G-1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. EtxB(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8+ T lymphocytes.

L6 ANSWER 28 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12
AN 1996:107787 BIOSIS
DN PREV199698679922
TI Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: Receptor binding is essential and induces differential modulation of lymphocyte subsets.
AU Nashar, Toufic O. (1); Webb, Helen M.; Eaglestone, Simon; **Williams, Neil A.**; Hirst, Timothy R.
CS (1) Research School Biosciences, University Kent, Canterbury, Kent CTG2 7NJ UK
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 1, pp. 226-230.
ISSN: 0027-8424.
DT Article
LA English
AB The importance of receptor binding in the potent immunogenicity of Escherichia coli heat-labile enterotoxin B subunit (EtxB) was tested by comparing its immunogical properties with those of a receptor binding mutant, EtxB(G33D). Subcutaneous immunization of EtxB(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type EtxB, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two protèins induced strikingly different effects on lymphocyte cultures in vitro. EtxB, in comparison with EtxB(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma. These data indicate that EtxB exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

L6 ANSWER 29 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1995:863298 CAPLUS
TI Platinum catalyzed regioselective ortho-silylation of benzylideneamines via intramolecular C-H activation
AU **Williams, Neil A.**; Uchimaru, Yuko; Tanaka, Masato
SO J. Chem. Soc., Chem. Commun. (1995), (19), 2045
CODEN: JCCCAT; ISSN: 0022-4936
DT Journal; Errata
LA English

AB Unavailable

L6 ANSWER 30 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1995:629350 CAPLUS
DN 123:286129
TI Platinum catalyzed regioselective ortho-silylation of benzylideneamines via intramolecular C-H activation
AU **Williams, Neil A.**; Uchimaru, Yuko; Tanaka, Masato
CS Natl. Inst. Mater. Chem. Res., Ibaraki, 305, Japan
SO J. Chem. Soc., Chem. Commun. (1995), (11), 1129-30
CODEN: JCCCAT; ISSN: 0022-4936
DT Journal
LA English
OS CASREACT 123:286129
AB The Pt-P(OCH2)3CEt complex catalyzes the ortho-silylation of benzylideneamines with disilanes via intramol. C-H activation; both mono- and bis-silylated products are obtained.

L6 ANSWER 31 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 13
AN 1995:202134 BIOSIS
DN PREV199598216434
TI Immunohistochemical Detection of T-cell Subsets and Other Leukocytes in Paraffin-embedded Rat and Mouse Tissues with Monoclonal Antibodies.
AU Whiteland, Joanne L.; Nicholls, Susan M. (1); Shimeld, Carolyn; Easty, David L.; **Williams, Neil A.**; Hill, Terry J.
CS (1) Dep. Ophthalmol., Sch. Med. Sci., University Walk, Bristol BS8 1TD UK
SO Journal of Histochemistry and Cytochemistry, (1995) Vol. 43, No. 3, pp. 313-320.
ISSN: 0022-1554.
DT Article
LA English
AB We describe a method for immunohistochemical localization of T-cells, CD4+T-cells, CD8+T-cells, B-cells, activated lymphocytes, major histocompatibility complex (MHC) class II antigens, macrophages, dendritic cells, and granulocytes in rat and mouse tissue fixed in periodate-lysine-paraformaldehyde (PLP) and embedded in paraffin. Rat and mouse spleen and eyes were fixed in PLP for 18-24 hr, rapidly dehydrated, infiltrated under vacuum with paraffin at 54 degree C, sectioned, and stained with appropriate monoclonal antibodies (MAbs). Sections of PLP-fixed, paraffin-embedded spleen were compared with acetone-fixed frozen spleen sections with respect to morphology and staining quality. Nine of 10 MAbs to rat antigens and eight of nine MAbs to mouse antigens stained paraffin sections equally or more intensely than frozen sections. The two MAbs that showed weaker staining still gave good staining on paraffin sections. Paraffin-embedded rat and mouse eyes were easier to section serially than frozen eyes, showed superior morphology, and individually stained cells were readily identified. Therefore, a combination of PLP fixation and low-temperature paraffin embedding permits detection of the major types of immune cell in rat and mouse tissues while maintaining good morphology, particularly in diseased, damaged, or delicate tissues.

L6 ANSWER 32 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 14
AN 1995:157713 BIOSIS
DN PREV199598172013
TI Immunologically ignorant autoreactive T cells, epitope spreading and repertoire limitation.
AU Elson, Christopher J.; Barker, Robert N.; Thompson, Stephen J.; **Williams, Neil A.**
CS Univ. Bristol, Dep. Pathol. and Microbiol., Bristol BS8 1TD, England UK
SO Immunology Today, (1995) Vol. 16, No. 2, pp. 71-76.
ISSN: 0167-4919.

DT Article
LA English

L6 ANSWER 33 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1991:677715 CAPLUS
DN 115:277715
TI The in vitro production of cytokines by mucosal lymphocytes immunized by oral administration of keyhole limpet hemocyanin using cholera toxin as an adjuvant
AU Wilson, A. Douglas; Bailey, Michael; **Williams, Neil A.**; Stokes, Christopher R.
CS Sch. Vet. Sci., Univ. Bristol, Langford/Avon, BS18 7DU, UK
SO Eur. J. Immunol. (1991), 21(10), 2333-9
CODEN: EJIMAF; ISSN: 0014-2980
DT Journal
LA English
AB The in vitro prodn. of a variety of cytokines by lymphocytes isolated from spleen mesenteric lymph node (MLN), Peyer's patches (PP) and lamina propria (LP) was measured, after oral immunization with keyhole limpet hemocyanin using cholera toxin as an adjuvant. LP responses were characterized by very high levels of interleukin (IL) 4, IL 5 and IL 6 with lower levels of IL 2 and interferon-.gamma. (IFN-.gamma.). The PP had lower levels of IL 4, IL 5 and IL 6 than LP but higher levels of IL 2; IFN-.gamma. was only present at very low levels in this organ. The MLN had a pattern of cytokine prodn. similar to the PP but did produce IFN-.gamma.. The spleen produced all cytokines measured except IL 5. Antibody prodn. was characterized by IgA in the LP and PP but IgG was the dominant isotype in the spleen. The MLN was a poor source of antibody-producing cells. The results are interpreted to show that (a) the LP response to cholera toxin/keyhole limpet hemocyanin is dominated by Th2-type cytokines compared to a lower prodn. of Th1 type and (b) that the PP has responses typical of an organ with a high proportion of resting lymphocytes which develop mainly into Th2-types cells. The spleen is less dominated by Th2-type cytokines than the mucosal sites and this difference is paralleled by IgA antibody prodn. at the mucosal sites and IgG antibody dominance in the spleen.

L6 ANSWER 34 OF 34 CAPLUS COPYRIGHT 2002 ACS
AN 1992:225 CAPLUS
DN 116:225
TI Effects on murine epidermal Langerhans cells of drugs known to cause recrudescent herpes simplex virus infection in a mouse model
AU **Williams, Neil A.**; Hill, Terry J.
CS Dep. Pathol. Microbiol., Sch. Med. Sci., Bristol, BS8 1TD, UK
SO J. Invest. Dermatol. (1991), 97(5), 933-7
CODEN: JIDEAE; ISSN: 0022-202X
DT Journal
LA English
AB A no. of agents have been shown to alter the latent state of herpes simplex virus in murine sensory ganglia. However, it seems that effective triggers of recrudescent disease must act not only to reactivate latent herpes simplex 1 (HSV) infection, but also to create a favorable environment in the skin for vital replication. The possibility that alteration of the local Langerhans cell population is one way in which effective triggers of recrudescence may act has been investigated. Of the agents tested, which affect latent HSV, only DMSO significantly altered the nos. of ATPase-bearing Langerhans cells in the epidermis, maximally reducing their d. by 83% in 48 h. Xylene and retinoic acid had no discernible effect on nos. of ATPase-staining cells over the 4 d tested. However, the extent to which agents reduced ATPase-staining cell nos. did not correlate with their ability to affect the antigen-presenting capacity of the cells in HSV-specific T-cell proliferative assays in vitro. Xylene and retinoic acid markedly reduced the accessory cell functio nof epidermal cell suspensions, whereas DMSO had no effect.